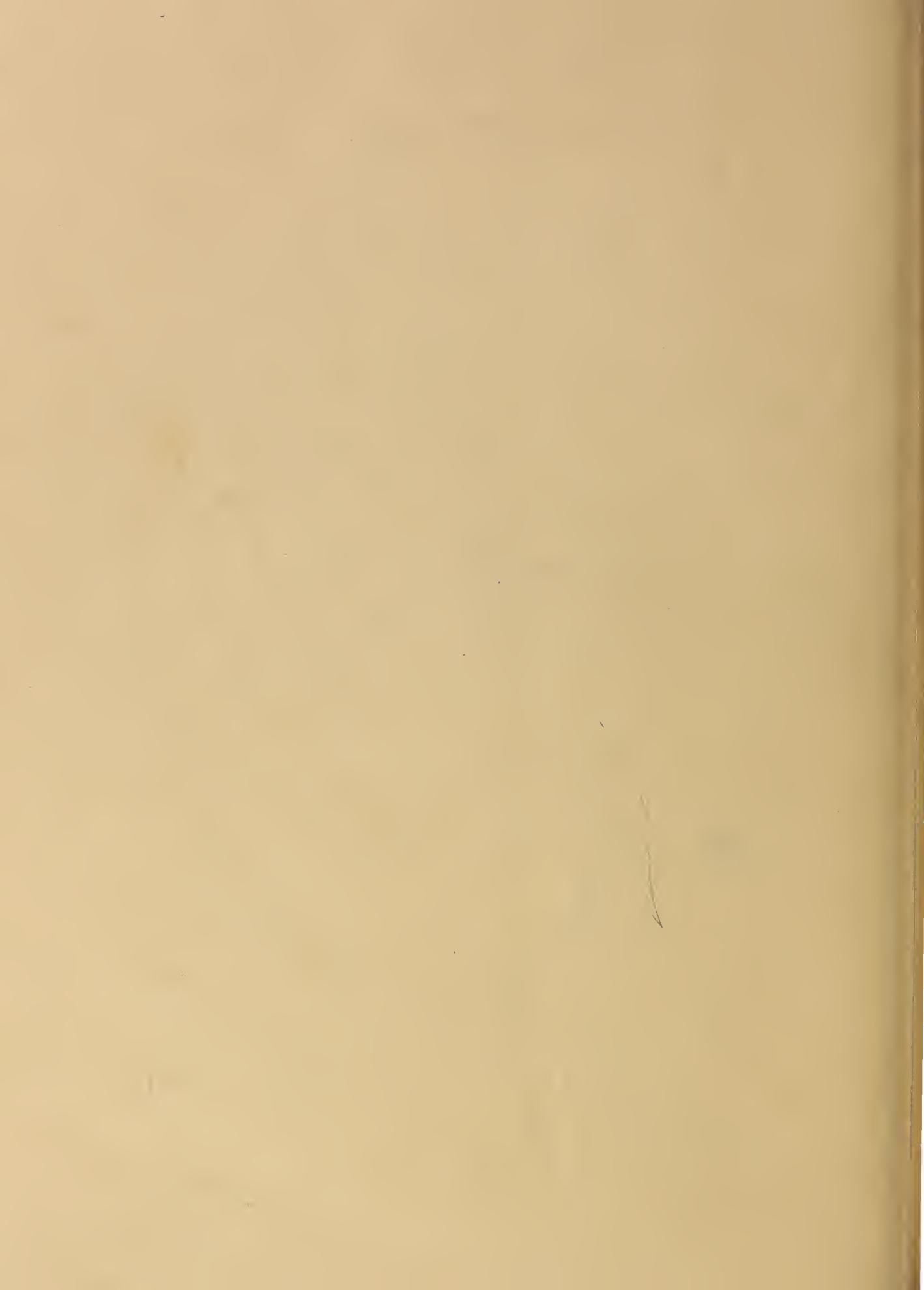


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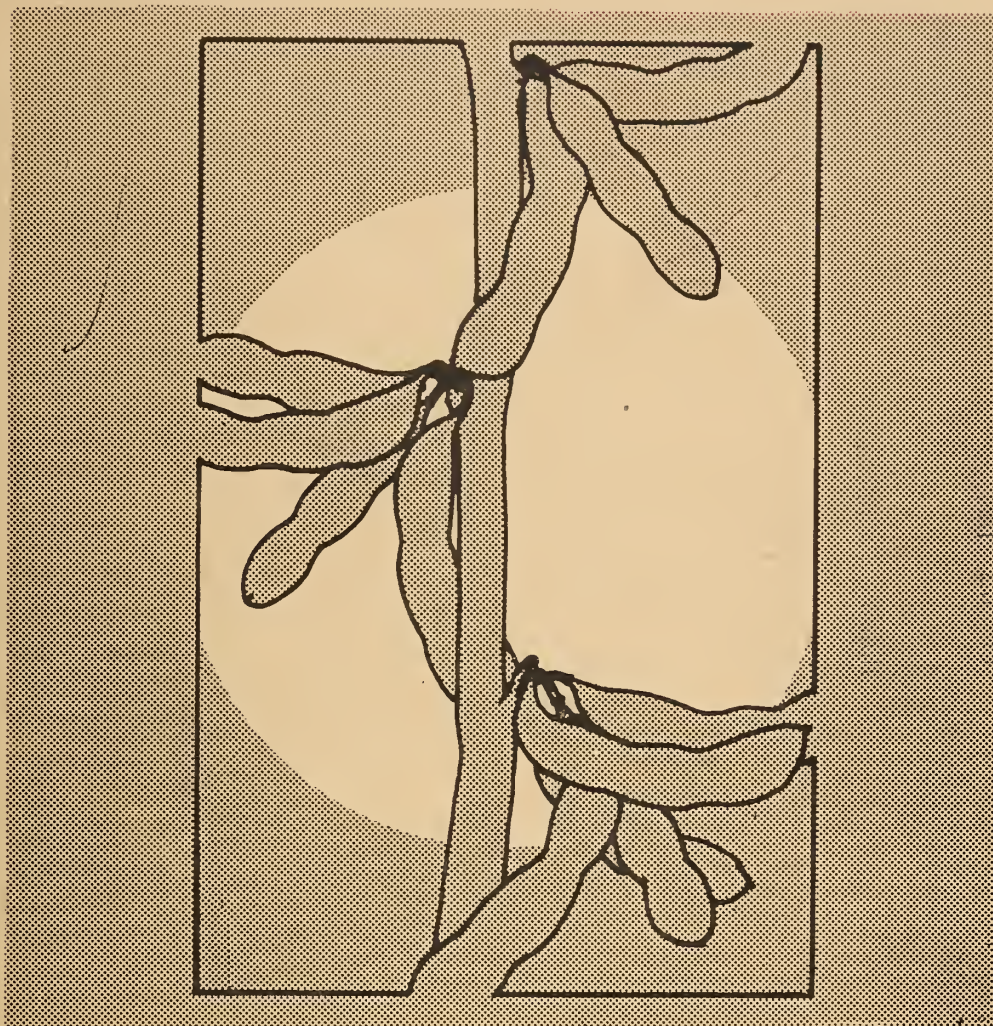
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Soybean Genetics Newsletter

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Volume 16

April 1989

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publications without the consent of the respective authors.

Agricultural Research Service - USDA
Department of Agronomy
and Department of Genetics
Iowa State University
Ames, Iowa 50011



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FOREWORD

Volume I of the Soybean Genetics Newsletter was issued in April, 1974. The articles in volume 16 give an indication of the international nature of the Soybean Genetics Newsletter.

The Newsletter is free upon request. Our costs of publishing the Newsletter continue to increase. We are considering several mechanisms to secure funds to continue publication. We will report our decision in the annual request for contributions for Volume 17, to be mailed to all subscribers in October.

Our typist for previous newsletters, Mrs. Donna Gladon, has returned to Iowa State University. Our special thanks go to Holly Heer, who has been the typist, proof reader, and organizer of this Newsletter. Thanks again, Holly, for your outstanding efforts. The capable assistance of Laurie Amberger, Sandra Benavente, Dana Schaulis, Laura Sellner, and Zhang Fan is gratefully acknowledged.

Reid G. Palmer, editor

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SOYBEAN GENETICS COMMITTEE REPORT - FEBRUARY 1989

Minutes of the Meeting

The Soybean Genetics Committee met February 20, 1989, in conjunction with the Soybean Breeders Workshop in Memphis, TN.

Committee members in attendance were G. R. Bowers, R. I. Buzzell, X. Delannay, T. E. Devine, B. A. McBlain, R. L. Nelson, and J. H. Orf. Also present were R. L. Bernard, L. Mansur, and J. VanHerk. T. C. Kilen and J. R. Wilcox were elected to three-year terms, and B. A. McBlain was elected chairman. Current committee members and the expiration dates of their terms are as follows:

G. R. Bowers (1990)
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Route 7, Box 999
Beaumont, TX 77706
(409) 752-2741

T. E. Devine (1991)
PMBL, B-008, Rm 101
BARC-West, Beltsville, MD 10705
(301) 344-4375

T. C. Kilen (1992)
USRA ARS
PO Box 196
Stoneville, MS 38776
(601) 686-9311

B. A. McBlain (1990) Chairman
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B. D. Rennie (1991)
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Purdue University
W. Lafayette, IN 47907
(317) 494-8074

Procedures: As in the past, manuscripts concerning qualitative genetics interpretation and gene symbols should be sent to the chairman for review. The Committee will carry out the review process as indicated in the 1988 Soybean Genetics Newsletter 15:3.

Assignment/Approval of Gene Symbols: If gene symbols are being assigned in genetic studies where the material is from induced mutants, variants from heterogeneous populations, or from transgenic changes, then the authors should deposit representative genetic material in the Genetic Type Collection. Dr. R. L. Nelson is Curator for all maturity groups. An application form for this is on page 6.

The Committee amended the "Guidelines on the Evidence Necessary for the Assignment of Gene Symbols", Soybean Genetics Newsletter 14:7, 1987, to include the following:

8. In those cases where a proprietary mutant or transgenic line is not placed in the USDA Germplasm Collection, then such a line must be available from the originator by researchers for research purposes; the originator's supplier should be indicated in the paper. In addition, such lines should be placed in long-term storage at Ft. Collins to safeguard against loss in the future.

Gene symbols will be approved only in cases where the relevant material is available for distribution to researchers. The Committee encourages authors not to assign any symbol when they are doing genetic work on material that will not be made available. (Publication of genetic interpretations does not depend on symbols, in most cases). The purpose of assigning a symbol is to ensure constancy when others use the material for subsequent studies. If the material is not made available, a symbol is unnecessary.

Summaries for the Past Year: A list of the soybean gene symbols approved from March 1987 to February 1988 is given in Table 1. Previously approved genes that have been published recently are given in Table 2.

It has been brought to the attention of the Committee that the gene symbols Rcs3 and rcs3 have been published (1;9) but were inadvertently omitted from the 1987 list of Approved Soybean Gene Symbols (8). These symbols were approved by the Committee February 15, 1983.

Anyone knowing of soybean gene symbols used prior to 1987 that do not occur in the Gene Symbol Index, pages 192-196 of the 1987 Soybean Monograph (14) please advise the Soybean Genetics Committee.

R. I. Buzzell
Past Chairman

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15. ____ and J. F. Cavins. 1987. *J. Hered.* 78:410.

Table 1. Soybean gene symbols approved March 1988 - February 1989

Date	Authors	Trait	Genes
June 15, 1988	Willmot & Nickell	Brown stem rot R	<u>Rbs3</u> <u>rbs3</u>
July 13, 1988	Skorupska & Palmer	Male sterility	<u>Ms6</u> <u>ms6</u>
Sept. 1, 1988	Groose et al.	Mutable flower	<u>w4-m</u>
Oct 3, 1988	Buzzell & Tu	SMV reaction	<u>Rsv3</u> <u>rsv3</u>
Feb. 13, 1989	Rennie & Tanner	Linolenic acid	<u>fan</u> (PI 123440)
Feb. 16, 1989	Rennie & Tanner	Linolenic acid	<u>fan</u> (A5)
Feb 17, 1989	Lim	Downy mildew R to race 2	<u>Rpm2</u> <u>rpm2</u>
Feb. 20, 1989	Sebastian et al.	Sulfonylurea R	<u>Als1</u> <u>als1</u>

Table 2. Annual list of recent publications having approved gene symbols.

Gene	Strain	Phenotype	References
<u>Enp-a</u>	Emerald	Endopeptidase band Rf 0.39	4
<u>Enp-b</u>	A.K. (FC 30.671)	Band Rf 0.42	4
<u>Enp-c</u>	PI 504.287	Band Rf 0.46	4
<u>Estl-a</u>	AV68*; Aoda	Carboxylesterase Rf 0.06	2
<u>Estl-b</u>	T289; Beeson	Fast band Rf 0.11	2
<u>fan</u> (C1640)	T280	Lower level of linolenic acid in seed	15,10
<u>fan</u> (PI 361088B)		Allele similar to above	10
<u>Fap1</u>	Century	Intermediate palmitic acid	6
<u>fap1</u>	EMS>C1726	Lowered palmitic acid	6
<u>Fap2</u>	Century	Intermediate palmitic acid	6
<u>fap2</u>	EMS>C1727	Increased palmitic acid	6
<u>Fle</u>	A.K.(FC 30.671)c**	Fluorescent esterase	5
<u>fle</u>	Emerald; c	Band 1 absent	5
<u>ms1</u> (Danbury)	T290	Male sterile	11
<u>ms3</u> (Flanagan)	T284	Male sterile	7
<u>ms3</u> (Plainview)	T291	Male sterile	12
<u>ms4</u> (Fisher)	T292	Male sterile	13
<u>Pgil-a</u>	PI 424.032	Phosphoglucose isomerase Band 6 Rf 0.58	3
<u>Pgil-b</u>	Lindarin	Band 6 Rf 0.67	3
<u>pgil</u>	PI 157.401	Null band 6	3
<u>Pgi2</u>	Lindarin	Dominant band 1	3
<u>pgi2</u>	PI 157.401	Null band 1	3
<u>Pgi3-a</u>	PI 407.260	Band 3 Rf 0.42	3
<u>Pgi3-b</u>	AV68*	Band 3 Rf 0.45	3
<u>Rcs3</u>	Davis	Frogeye R to races 2 & 5	1,9
<u>rsc3</u>	Kent	Susceptible to race 5	1,9

>=mutant induced by chemical mutagen

*AV68 is from Asian Vegetable Research and Development Center, Taiwan.

**c indicates that the gene commonly occurs in most varieties.

APPLICATION FOR ENTRY INTO THE SOYBEAN GENETIC TYPE COLLECTION

Date: _____
 Submitted by: _____
 Address _____

Return to:
 R. L. Nelson, Curator
 USDA Soybean Germplasm Collection
 Department of Agronomy
 University of Illinois
 W-321 Turner Hall
 1102 South Goodwin Ave.
 Urbana, IL 61801 USA

Strain designation: _____ T-number assigned: _____

Genotype: _____

Phenotype: _____
 (List the gene(s) and a description of the phenotype of the trait)

Parental origin: _____

When and where found and by whom: _____

(Include year, location, institution, and name of individual making find or in charge of research.)

Description: Maturity Group _____ Stem termination _____ Flower color _____

Pubescence color _____ Pubescence type _____ Pod color _____

Seed coat luster and color _____ Hilum color _____ Other _____

Special instructions for growing or maintenance, if any: _____

Literature reference: _____

(List the references(s) that first and best describe the discovery and inheritance of the trait. Please send relevant reprints to curator.)

Date seedlot received at Urbana: _____

SOYBEAN GENETICS COMMITTEE .

NOMENCLATURE SYSTEM FOR TRANSGENIC GENES //

As transformation technology is making good progress in various crops, it won't be very long before transgenic plants are commonly used by breeders, geneticists and other researchers, both public and private. As those plants will carry new genes located on various parts of the chromosomes, it becomes necessary to devise a general nomenclature system that will accurately describe the genetic makeup of the new plants. This system should be compatible with the existing conventions for naming traditional genes, but should also address the added complexity brought about by the genetic engineering process.

I would like first to describe some of the unique features of transgenic genes, which distinguish them from traditional genes:

1. Characterization of the gene sequences:

A traditional gene is usually defined by its phenotypic expression and the symbol used usually refers to that phenotype. Beyond the inheritance pattern and possibly the linkage group, little else is usually known, especially in terms of DNA sequence.

In the case of bioengineered genes, a particular phenotype is also obtained, but a great deal of information is known in terms of DNA sequence, and multiple variations in possible combinations of promoters, coding sequences and 3' ends can be used, and still lead to generally similar phenotypes. It is therefore important to characterize the gene more precisely than just by its phenotype, and to identify the particular DNA sequence used.

2. Localization of the introduced gene:

All transformation processes currently in use result in blind insertion of a gene construct into seemingly random parts of the chromosomes. This means that each independent transformation event will result in a different locus, and that there is a very large number of potential loci for each gene. Once inserted, the gene will be stably transmitted to the offspring in a Mendelian fashion as a dominant gene, so that it is very important to identify accurately the different loci formed in independent transgenic lines, even if they all carry the same gene construct. If independent transgenic lines are crossed, the offspring will segregate, with a potential loss of the new genes if the breeder is unaware of the locus differences between the lines. Another important point is that insertions in various parts of the genome will result in slight

differences in expression of the gene, and potential inactivation of neighboring genes, which reinforces the necessity to correctly identify the independent loci.

3. Multiple genes on single loci:

Even if a transformed plant is usually described by its main agronomic characteristic (insect tolerant, etc.), the construct used in the transformation process is always made up of multiple genes, typically at least a selectable (kanamycin resistance, etc.) or scoreable (nopaline, GUS, etc.) marker besides the agronomic gene. There may also soon be gene constructs with a combination of agronomic genes (insect resistance + herbicide resistance, etc.). All those multiple genes are transmitted to the offspring as a block, as a single gene, so that they should be assigned a single gene symbol.

4. Introduction of the same gene constructs in different crops:

The same gene constructs can (and will) be inserted in widely different crops that cannot be crossed naturally, such as tomatoes, soybeans, canola, corn, etc. Traditionally, there has not been any need for coordination among the geneticists of all those crops because the genes could be used only in the crop where they had been identified. Nomenclature systems are also quite different among crops. In the case of transgenic genes, it will be useful to devise a common system so that the same gene can be named in a similar way in various crops.

5. Public vs. private transgenic lines:

The majority of transformation efforts are currently done by private institutions and a large number of constructs or transgenic lines may never be released. When they are released, their commercial use will probably be limited to specific seed companies, at least until patent expiration. However, even those commercially restricted transgenic lines may sometimes be used by public or private institutions for research purposes, and the results will be published, as is now done commonly with proprietary chemicals from the pesticide and other businesses. The need for a generalized naming system therefore extends to both privately and publicly developed lines.

Since soybean transformation is a reality now, the Soybean Genetics Committee has considered a nomenclature system for future transgenic genes. Transgenic genes introduced into soybean plants should be named in the following format:

[construct name]_n

where the name of the construct (using the system used by molecular

biologists) is put inside brackets, and n is a number corresponding to a particular insertion event, and therefore identifies a particular locus.

For instance, if the construct pMON200 has been introduced independently into five soybean plants that have been determined to be useful for future research or commercialization, the five plants and their progenies would carry the following genes, respectively:

[pMON200]₁

[pMON200]₂

[pMON200]₃

[pMON200]₄

[pMON200]₅

The numbering should continue where it left off for any additional plants produced carrying the same construct. No symbol is necessary for the corresponding null allele of each of these loci, or a hyphen or other symbol can be used.

This system seems to address all the concerns expressed earlier. The construct name exactly identifies the particular gene sequence and gene combination, and its phenotypic characteristics can easily be obtained from a frequently updated construct list. The information in that construct list may be more or less detailed, depending on its proprietary nature, but should be sufficient for use in most genetic, breeding, or physiology studies. Unless the construct is made publicly available, the originator of a construct should take charge of coordinating the assignment of loci numbers by its users. The Soybean Genetics Committee will maintain a list of genes symbols for publicly available constructs or transgenic plants. To avoid unnecessarily large numbers of loci for the same construct, only the transgenic lines retained for further use should receive a locus number.

Nomenclature system for transgenic genes adopted by the Soybean Genetics Committee February 20, 1989.

Xavier Delannay
Monsanto Company
St. Louis, MO.

J
SOYBEAN GERMPLOSM CROP ADVISORY COMMITTEE REPORT

The Soybean Germplasm Crop Advisory Committee (CAC) held its annual meeting February 20, 1989, at the Soybean Breeders' Workshop in Memphis, TN. Eleven of the 14 members were in attendance. Those elected to three-year terms were: T. Scott Abney, USDA-ARS, W. Lafayette, IN; Dennis B. Egli, University of Kentucky, Lexington, KY; and J. Grover Shannon, Delta and Pine Land Co., Scott, MS.

Updates on the northern and southern portions of the USDA Soybean Germplasm Collection were given by Randall Nelson, Calton Edwards, and Edgar Hartwig. Reports on those collections are presented on pages 16 and 17 of this volume.

The Soybean Germplasm CAC By-Laws were published in the Soybean Genetics Newsletter 13:31-32, 1986. By-law changes concerning the length of service for the chairperson and vice-chairperson, and the establishment of subcommittees and ad hoc committees were approved by a majority vote of the committee members. These changes are included in the by-laws published on page 14.

The establishment of a collection of privately developed soybean varieties was discussed. The collection would be made up of privately developed varieties no longer offered for sale. The committee approved the concept of such a collection. The procedure for nominating varieties for this collection will be developed by Randall Nelson.

Members of the CAC discussed the desirability of more widely publicizing the collections that are available for research purposes. Newsletters published by various scientific societies were suggested as vehicles to reach a wider audience. There is a need to provide information on the soybean germplasm material and the names and addresses of persons to whom requests should be made. The curators will develop an appropriate information statement for the newsletters.

In response to a memo from Paul S. Fitzgerald, the CAC discussed the possibility of greater opportunities for scientific exchange between the U.S. and the U.S.S.R. The CAC members believe that the greatest soybean germplasm diversity exists in the far eastern U.S.S.R., and that soybean samples from that area would enhance our collection. The CAC chairperson has responded to Dr. Fitzgerald's memo, indicating that the committee supports an atmosphere of scientific exchange that will mutually benefit the two countries.

Randall Nelson reported on progress in U.S.-China cooperative research. In 1988, a cooperative variety test project was initiated between the two countries. This test will be repeated in 1989. Opportunities for cooperative research for selected specific traits were discussed. Protein content and resistance to viruses, soybean cyst nematode, insects, and drought are possible traits that would mutually benefit the two countries.

Printouts of available information on soybean germplasm through the Germplasm Resources Information Network (GRIN) was supplied by Mark Bohning. The committee members were invited to inspect the printouts and to inform people in their disciplines concerning the availability of this information. The printouts were also on display for people attending the general session of breeders and plant pathologists.

At the request of Allan K. Stoner, the committee discussed the feasibility of initiating a core collection for soybean. Members of the CAC believe that the core concept may be useful in terms of improving the evaluation and utilization of germplasm collections that are large and not well characterized. However, the soybean collection is relatively small, and has been extensively evaluated. This information has been entered into GRIN for the entire collection. Theoretically, it should be possible to define a core that would represent the majority of the genetic variation in the collection. However, the probability that rare mutations would be excluded in the core group, or that genetic diversity for a given trait would not be adequately represented is extremely high. In addition, such an approach would preclude maximal use of the GRIN system. Therefore, the committee believes that the entire soybean collection, which in reality is a working collection, should be considered as the core at this time. To enhance evaluation and utilization of accessions, very specific subcore groups could be established on specific requests and tailored to meet the needs of a requestor from the information in GRIN. A subcore population of less than 10% should then adequately sample the collection, through a directed means, for any given objective. The committee realizes that if the total collection becomes so large that it would not be feasible to supply the requestor with a sample of every accession in the collection, the core concept should be reevaluated.

Committee members briefly discussed patenting of enhanced germplasm. The members believe that publicly funded breeding programs have an obligation to encourage the development and facilitate the utilization of soybean germplasm. Restrictive patents are counter-productive to that effort. Widespread use of such patents based upon traits that may exist in nature could eventually negate the utility of the soybean germplasm collections and restrict efforts to maintain genetic diversity in a useful manner. Varieties and certain genetically engineered genotypes may be legitimately patentable. However, restrictive patents for germplasm that is enhanced by traditional breeding methods is not in the best interest of the National Plant Germplasm System.

Richard Bernard retired from the CAC and was recognized for actively serving the committee since its inception. Richard Wilson was thanked for his six consecutive years of active service on the CAC.

Following are the current committee members, addresses, phone numbers, areas of representation, and years of service:

Name	Address	Area of representation	Years of service
T. Scott Abney (317)494-4650	USDA-ARS; Dept of Botany & Plant Path., Purdue Univ., W. Lafayette, IN 47907	Plant pathology	1986-1989 1989-1992
Dennis B. Egli (606)257-7310	Dept. of Agron., Univ. of Kentucky Lexington, KY 40506	Physiology	1989-1992
Edgar E. Hartwig (601)686-9311	USDA-ARS Soybean Prod. Res. PO Box 196 Stoneville, MS 38776	USDA Germplasm Collection	ex officio
Kuell Hinson (904)392-6188	USDA-ARS Dept of Agron., Univ. of Florida 1 FAA Bldg. 63 Gainesville, FL 32611	Public breeding South	1984-1987 1987-1990
Clark Jennings (319)234-0335	Pioneer Hi-Bred Int'l. 3261 W. Airline Hwy. Waterloo, IA 50703	Private breeding North	1984-1987 1987-1990
Thomas C. Kilen (601)686-9311	USDA-ARS Soybean Prod. Res. PO Box 196 Stoneville, MS 38776	USDA Germplasm Collection	ex officio
Philip Miller (202)344-2725	USDA-ARS Beltsville Agric. Res. Ctr., Bldg. 005, BARC- West, Beltsville, MD 20705	USDA National Program staff	ex officio
Randall Nelson (217)244-4346	USDA-ARS Dept. of Agron., Univ. of Illinois 1102 S. Goodwin Urbana, IL 61801	USDA Germplasm Collection	ex officio
J. H. Orf (612)625-8275	Dept. of Agron. & Plant Genetics Univ. of Minnesota St. Paul, MN 55108	Public breeding North	1985-1988 1988-1991
Reid G. Palmer (515)294-7378	USDA-ARS G-301 Agronomy Iowa State University Ames, IA 50011	Cytogenetics & Molecular Genetics	1985-1988 1988-1991

Name	Address	Area of representation	Years of service
Donald P. Schmitt (919)737-3905	Dept. of Plant Path. Box 7631 North Carolina State University Raleigh, NC 27695-7631	Nematology	1987-1990
J. Grover Shannon (601)742-3351	Delta & Pine Land Co. Scott, MS 38772	Private breeding South	1986-1989 1989-1992
Lavone Lambert (601)686-2311	USDA-ARS Southern Field Crop Insect Mgmt. Lab. PO Box 346 Stoneville MS 38776	Entomology	1988-1991

Thomas C. Kilen was reelected as chairman of the committee and Clark Jennings was reelected vice-chairman. Both will serve one-year terms.

Thomas C. Kilen, Chairman
Soybean Germplasm Crop
Advisory Committee

SOYBEAN GERMPLASM CROP ADVISORY COMMITTEE BY-LAWS

Membership

The Soybean Germplasm Crop Advisory Committee will consist of 14 members. The curators of the northern and southern portion of the collection, the research geneticists working with the germplasm collection at each location, and a representative from the National Program Staff will serve as ex officio members. All ex officio members will have full voting privileges and may hold committee offices.

The remaining nine committee members will be elected to the committee to represent various geographical and/or research areas as follows:

1. Private breeder, north
2. Private breeder, south
3. Public breeder, north
4. Public breeder, south
5. Pathologist or nematologist
6. Pathologist or nematologist
7. Entomologist
8. Physiologist or biochemist
9. Cytogeneticist or molecular geneticist

Terms of Office

Committee members will be elected to three-year terms and may serve no more than 2 consecutive terms. After an absence of at least one year from the committee, a former two-term member is eligible for membership again. Terms will begin following the annual meeting held in conjunction with the Soybean Breeders' Workshop in late February.

Committee Officers

The committee shall have two elected officers, chairperson and vice-chairperson. Each officer will be elected to a one-year term at the committee's annual meeting to serve the following year and may serve no more than five terms out of any eight-year period.

The duties of the committee chairperson include coordinating annual elections, notifying members of meetings, chairing meetings, and other duties as necessary to fulfill the committee's responsibilities. The vice-chairperson shall record the proceedings of all meetings and assist the chairperson as requested.

Sub-committees

Standing sub-committees shall be established and approved by a majority vote of the committee. Ad hoc sub-committees shall be established as needed by the chairperson.

Elections

Each year three members will be elected in the following manner:

- Year 1: Entomologist
 Northern breeder, public
 Cytogeneticist or molecular geneticist

- Year 2. Southern breeder, private
 Physiologist
 Pathologist or nematologist (position A)
- Year 3. Northern breeder, private
 Southern breeder, public
 Pathologist or nematologist (position B)

By November 1 of each year, the chairperson will send a request for nominations for each position for which the incumbent's term expires the following February. These requests will be sent to those whose discipline and geographical areas are the same as the qualifications for the open committee position. All nominations must be received by the chairperson by November 30. If more than two nominations are received for any position, the chairperson will send all nominations to the committee and each member may vote for two candidates for each position. The ballots from this primary election must be sent by December 7 and returned to the chairperson by January 7. A final ballot with the top two candidates for each position will be sent to the committee by January 10. These ballots must be returned to the chairperson by January 27. The chairperson will then notify the newly elected members so that they may attend the annual meeting in late February. The newly elected members will officially begin their terms after that meeting but will be invited to attend as observers.

Rule Changes

These rules may be amended by a majority vote (8) of the committee members.

USDA SOUTHERN SOYBEAN GERMPLASM COLLECTION REPORT

FEBRUARY 1989

	Total entries	Total entries	Total entries	Total entries
Maturity	1984	1985	1987	1988
V	1549	1550	1550	1813
VI	482	486	487	787
VII	346	349	351	449
VIII	297	303	308	352
IX	131	143	149	149
X	154	158	165	165
	<u>2959</u>	<u>2989</u>	<u>3010</u>	<u>3715</u>

40 new additions were pure lined in 1988. Seed will be available after increase in 1989. These will be added to GRIN in 1989.

Stoneville, MS will grow 700+ lines of Germplasm Group V in 1989 for increase.

Stoneville will grow 18 lines of 4 plants each for single row selection in 1989.

1988 seed requests filled: 912 packets, to 29 countries and 3614 packets to 27 states. Total requests 166, total packets 4553.

705 additional germplasm lines were added to the collection at Stoneville after January 1987 and are available to users of the GRIN system in 1989.

C. E. Edwards, Jr.
E. E. Hartwig

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USDA NORTHERN SOYBEAN GERMPLASM COLLECTION REPORT

February 1989

On December 31, 1988, Richard L. Bernard retired from the USDA and from his position as curator of the USDA Northern Soybean Collection after nearly 35 years of service. During his tenure the collection at Urbana, IL has grown from less than 2000 to more than 9000 accessions. But numbers alone do not adequately describe his contribution. His genetic research on the collection, his willingness to assist others in utilizing the collection, and the high standards that he has set for documenting and maintaining the collection have greatly contributed to soybean research in the United States and around the world. Dr. Bernard's contributions will not cease with his retirement. The results of his commitment to soybean germplasm will continue to have an impact for as long as this collection is maintained.

In October, 1988, Gail A. Juvik, Assistant Curator, transferred to the Germplasm Resources Information Network staff at Beltsville, MD. She became the first Assistant Curator of the collection in 1983. Her leadership in this position and her contributions to the management of the collection are greatly appreciated.

In 1988, 9043 seedlots were sent from Urbana. Sixteen percent went to private research organizations and the remaining to public institutions. Eighty four percent of all requests were domestic.

Seed increases were grown in the field for 1412 G. max accessions and, through a cooperative agreement with Theodore Hymowitz, University of Illinois, 157 perennial Glycine accessions.

Another general evaluation of G. max accessions between PI 490.765 and PI 507.573 was begun in 1988. Jim Orf, University of Minnesota, is evaluating 43 accessions in Maturity Groups 0 and earlier. At Urbana, 16, 78, 149, and 270 accessions in Groups I, II, III, and IV, respectively, are being evaluated. Because of the abnormally dry conditions in 1988, we are planning to grow this evaluation for an additional two years.

New soybean accessions were added to the collection from the U.S.S.R. (63), Taiwan (26), S. Korea (19), and Japan (1). These are available for distribution. In addition, 40 soybean seedlots were grown for the first time from China (30), Yugoslavia (5), U.S.S.R. (2), Taiwan (2), and S. Korea (1). We added 96 new accessions of wild soybean to the collection from the U.S.S.R. (83) and Japan (13) and grew 60 new wild soybean seedlots from U.S.S.R. (56) and Japan (4). All accessions grown for the first time in 1988 will be available for distribution after harvest in 1989.

Two new germplasm publications were widely distributed to soybean scientists in the U.S. and to germplasm institutions around the world:

Nelson, R.L., P.J. Amdor, J.H. Orf and J.F. Cavins. 1988.

Evaluation of the USDA Soybean Germplasm Collection: Maturity Groups III to IV (PI 427.136 to PI 445.845). USDA Technical Bulletin No. 1726. 185 pp.

Bernard, R.L., G.A. Juvik, E.E. Hartwig, and C.E. Edwards, Jr. 1988.

Origins and Pedigrees of Public Soybean Varieties in the United States and Canada. USDA Technical Bulletin 1746. 68 pp.

If you did not receive copies of these publications and would like to be added to our mailing list for future germplasm publications, requests can be sent to:

Randall Nelson, USDA ARS
Department of Agronomy
University of Illinois
1102 S. Goodwin Ave.
Urbana, IL 661801

WORLD BIBLIOGRAPHY OF SOYBEAN ENTOMOLOGY

Publication of the "World Bibliography of Soybean Entomology", in two volumes, represents the culmination of nearly 10 years of intensive bibliographic research. Over 5,000 documents have been collected, classified using a thesaurus of hierarchical key words, and processed for electronic storage and retrieval.

Volume I contains an introduction with a list of major pest species in each of six production regions of the world and a bibliometric study of the literature. Criteria for inclusion of documents and the structure of the bibliography are explained in the introduction. Bibliographic entries appear in alphabetical order by author(s). Each entry is followed by a string of key words that reflects the subject contents. These key words are also used in the subject indexes in Volume II.

Volume II contains six indexes that refer to the sequentially numbered entries:

1. Author index. An alphabetical list of all authors. Users can retrieve the entire contribution of a given author, whether that author appears as the senior or junior author.
2. Geographic index. A list of the countries in which the research was done or that are mentioned in the entries.
3. Language index. A list by language of all entries, excluding those printed in English.
4. Host plant index. A hierarchical list of plants (family and genus) mentioned in the entries.
5. Taxonomic index. A hierarchical list of arthropods (order, family, and genus).
6. General descriptor index. A list of subject descriptors hierarchically organized. A subindex for these descriptors is organized alphabetically.

Taken together, the geographic and the language indexes indicate the international scope of the bibliography.

The host plant, arthropod taxonomic, and general descriptors indexes

are hierarchically organized. To facilitate use of the hierarchical indexes there is an alphabetic index of taxa and general descriptors, each followed by the higher level hierarchies and the page of the index volume where the descriptor appears. Thus, in searching for "Cerotoma trifurcata" one will find in the alphabetical index, the entry: Cerotoma trifurcata, Coleoptera: Chrysomelidae, p. 68. On page 68, Cerotoma trifurcata appears followed by the numbers of the citations that contain reference to that species. The indexes are a unique feature of this bibliography. Volume II also includes lists of abbreviations for all periodical titles in the bibliography.

The "World Bibliography of Soybean Entomology" has been compiled as part of the Soybean Insect Research Information Center (SIRIC) operations. SIRIC is supported by the University of Illinois Agricultural Experiment Station, the Illinois Natural History Survey and the American Soybean Association. Copies of the bibliography are being sold by the University of Illinois, Office of Agricultural Publications, 67 Mumford Hall, 1301 W. Gregory Drive, Urbana, IL 61801.

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CONTRIBUTIONS WANTED FOR THE JOURNAL

"SOYBEAN SCIENCE"

The quarterly journal "Soybean Science" now is ready to accept manuscripts in English. Contributions of research papers, reviews, and notes on genetics, breeding, germplasm, physiology, ecology, pathology, entomology, cultural practices and management, and other disciplines related to soybeans are welcome.

No page fees will be charged and no author's remuneration will be paid. Fifty free copies of reprints will be sent to the author(s) for each accepted paper. Please contact the Editorial Board for detailed information at the following address:

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 1) Comparison of the fan alleles in Cl640 and the lines A5, PI 123440, and PI 361088B.

The fan allele, initially identified in Cl640 (Wilcox and Cavins, 1987), is associated with a large reduction in the linolenic acid level of soybean oil. Recently, there have been reports that indicate that an fan allele also is present in the low linolenic acid lines PI 361088B, A5, and PI 123440. This paper presents a comparison between the fan allele in Cl640, and the three other fan alleles. (Table 1 summarizes this comparison.)

Cl640, the source of the original fan allele, was derived by mutagenesis of the cultivar 'Corsoy' (Wilcox and Cavins, 1985). The fan (Cl640) allele is responsible for linolenic acid levels that range from 3.0 to 4.0%, compared with a level of 7.0 to 8.0% observed for seeds of 'Century' (Fan/Fan) (Wilcox and Cavins, 1987). The heterozygous genotype has an intermediate linolenic acid value, regardless of the direction of the cross (Wilcox and Cavins, 1985). The fan (Cl640) allele maintains a relatively low linolenic acid level at low temperatures (unpublished data).

PI 361088B is a plant introduction line that has been observed to have a linolenic acid level similar to Cl640 (Rennie et al., 1988). The fan allele in PI 361088B has been isolated from its original background, and has been compared to the allele in Cl640. The fan (PI 361088B) allele can not be distinguished readily from the fan (Cl640) allele in the F1 or F2 progeny of crosses (Rennie et al., 1988) or by exposure to cool temperatures (unpublished data).

A5 was created by mutagenesis of a line with moderate linolenic acid levels (Hammond and Fehr, 1983). Although Graef et al. (1988) indicated that linolenic acid levels in A5 were a quantitative trait, Rennie and Tanner (1989a) were able to demonstrate the presence of an fan allele by examining the F2 progeny of crosses involving A5 and N78-2245, a low linolenic acid line with the Fan allele (J. W. Burton, 1988, pers. comm.). The fan (A5) allele would appear to differ from the fan (Cl640) allele as the F1 progeny of crosses involving A5 have linolenic acid levels that are influenced by the maternal parent (Graef et al., 1988). In addition, the pattern of segregation in the F2 progeny of crosses involving standard lines with A5 are continuous (Graef et al., 1988) while the progeny of such crosses with Cl640 have a trimodal distribution (Wilcox and Cavins, 1985). The linolenic acid levels in A5 are more sensitive to exposure to cool temperatures, relative to Cl640 (unpublished data). Since the fan

(A5) allele has not been isolated from the other loci in this line, it is not clear if the fan (A5) allele alone is responsible for these differences.

PI 123440 is a plant introduction line that was isolated on the basis of low linolenic acid levels (J. W. Burton, 1987, pers. comm.). Rennie and Tanner (1989b) have indicated that PI 123440 possesses an fan allele. The linolenic acid levels of the F1 progeny of crosses involving PI 123440 are similar to levels observed with PI 361088B as a parent (Rennie and Tanner, 1989b). However, the pattern of segregation in the F2 progeny of crosses involving N78-2245 differ between PI 123440 and C1640 (J. W. Burton, 1988, pers. comm.). In addition, the linolenic acid levels of PI 123440 are higher at cool temperatures, relative to C1640 (unpublished data). The fan (PI 123440) allele has not been isolated from any modifiers that may be present in PI 123440, and so it is not clear if this allele is responsible for all observed differences.

Table 1. Summary of a comparison of the fan (C1640) allele with similar alleles in the soybean lines PI 361088B, A5, and PI 123440.

fan (C1640) vs. fan (PI 361088B0)

- 1) both show no maternal effect in F1.
 - 2) both show trimodal segregation pattern in F2.
 - 3) both show low linolenic acid levels at 15/12 C.
-

fan (C1640) vs. fan (A5)*

- 1) maternal effect in F1 with A5, none with C1640.
 - 2) different patterns in F2 progeny from crosses to standard lines.
 - 3) higher 18:3 level in A5 vs. C1640 at 15/12 C.
-

fan (C1640) vs. fan (PI 123440)*

- 1) both show no maternal effect in F1.
- 2) both show trimodal segregation pattern in F2 with standard lines; but different patterns in F2 with N78-2245.
- 3) higher 18:3 level in PI 123440 vs. C1640 at 15/12 C.

*Unknown modifier alleles may be masking the true phenotype associated with the fan (A5) or fan (PI 123440) alleles.

References

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- ____ and _____. 1989b. Genetic analysis of the low linolenic acid levels in the line PI 123440. Soybean Genet. Newsl. 16:25-26.
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J. W. Tanner

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2) Genetic analysis of low linolenic acid levels in the line PI 123440

The soybean plant introduction line PI 123440 has a low level of linolenic acid when grown under standard field conditions (Martin and Rinne, 1984). The line PI 361088B has a similar linolenic acid level and is known to possess an fan allele that is responsible for this phenotype (Rennie et al., 1988). The object of this work was to determine the genetic basis for the low linolenic acid level in PI 123440 relative to alleles at the Fan locus.

PI 123440 was crossed to PI 361088B, which is known to possess an fan allele, and to PI 291277, which is known to possess the Fan allele (unpublished data). PI 361088B was also crossed to PI 291277, for comparison purposes. The hybrid status of putative F1 seeds was determined by means of starch gel electrophoresis. The parent and F1 plants were allowed to develop under controlled temperature conditions in the growthroom at a temperature regime of 28/22 C with 16 h daylength. The parent, F1, and F2 seed from each of these crosses were assayed for fatty acid levels by the procedures described by Rennie et al. (1988).

The linolenic acid levels in the F1 progeny of the cross involving PI 291277 (Fan/Fan) and PI 361088B (fan/fan) was 5.5%. The level in the F1 progeny of the cross (PI 291277 x PI 123440) was 5.4% (Table 1). These levels are not different from the appropriate mid-parent value. The F1 progeny of (PI 361088B x PI 123440) had a level of 3.9% linolenic acid. This was not different from the mean of either parent (Table 1).

The linolenic acid levels of the F2 progeny of the cross (PI 291277 x PI 123440) segregated in a distinct trimodal pattern with classes: low,

3.1 to 4.4%; intermediate, 4.7 to 7.2%; and high, 7.3 to 9.7%. The ratio of 64 low : 133 intermediate : 61 high is consistent with a 1:2:1 ratio ($\chi^2 = 0.32$, $P < 0.70$). The mean of the F2 progeny was not different from the F1 level or the mid-parent level (Table 1).

The F2 progeny of the cross (PI 361088B x PI 123440) did not segregate for linolenic acid levels and had a continuous distribution. The levels ranged from 2.5% to 5.0% for the F2 progeny, which was not different from the range observed for PI 361088B alone (Rennie et al., 1988). The mean of the F2 progeny was not different from the mean of either parent.

The data presented are consistent with the line PI 123440 possessing an fan allele as the major factor influencing linolenic acid levels. In order to distinguish it from the other known alleles at this locus the allele in PI 123440 has been designated fan (PI 123440).

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Table 1. Comparison of mean fatty acid levels in PI 123440, PI 291277, PI 631088B, and the F1 or F2 progeny from crosses with these lines.

Material	N	16:0*	18:0	18:1	18:2	18:3
PI 123440	12	10.9	2.9	22.1	59.1	3.6
PI 291277	12	12.1	3.5	17.7	58.2	7.6
PI 361088B	15	11.7	3.3	18.2	61.0	3.9
(PI 291277 x PI 361088B) F1	3	12.8	3.9	10.8	56.3	5.5
(PI 291277 x PI 123440) F1	4	13.1	3.8	21.6	56.2	5.4
(PI 281277 x PI 123440) F2	258	11.7	3.5	18.6	60.1	5.6
(PI 361088B x PI 123440) F1	3	12.5	3.6	19.5	59.7	3.9
(PI 361088B x PI 123440) F2	251	11.8	3.7	19.9	61.5	3.8

*Palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3).

B. D. Rennie
J. W. Tanner

3) ²⁴⁵ Linkage assays with fatty acid loci.

A6 possesses the fas^a allele which results in a very high level of stearic acid (Graef et al., 1985) and also possesses the Ap-c and Idh1-b isozyme alleles. G-LLA-F3 is a Guelph low linolenic acid line, a selection from the F3 progeny of the cross (Maple Arrow x PI 361088B) known to possess the fan allele and the Ap-b and Idh1-a isozyme alleles. C1640 is known to possess the fan allele (Wilcox and Cavins, 1987) and the Aco4-b allele. N78-2245 possesses the Fan allele (J. W. Burton, 1988, pers. comm.) and the Aco4-a allele.

One F1 plant from each of the crosses (A6 x G-LLA-F3) and (N78-2245 x C1640) was grown in a controlled growthroom facility at 28/22 C and 16 h daylength. The F2 seed were assayed for fatty acids using the procedure described by Rennie et al. (1988). Electrophoretic analysis was conducted as described by Rennie et al. (1987).

In the progeny of (A6 x G-LLA-F3) the alleles were segregating in a pattern consistent with a 1:2:1 ratio for each locus (data not shown). The alleles at each of the locus pairs segregated in a pattern consistent with a 4:2:2:2:2:1:1:1:1 ratio, which indicates that each pair involves two independent loci.

In the F2 progeny of (N78-2245 x C1640) the alleles at the Fan locus segregated in a pattern consistent with a 3:1 ratio, while the alleles at the Aco4 locus segregated in a pattern consistent with a 1:2:1 ratio (data not shown). The alleles in the locus pair Aco4 - Fan segregated in a pattern consistent with a 6:3:3:2:1:1 ratio, which indicates that these are two independent loci.

There have been no previous reports of assays involving the locus pairs: Fan - Aco4, Fan - Ap, Fas - Ap, or Fas - Idh1.

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Table 1. The F2 segregation ratios of alleles from pairs of loci in the progeny of the cross (A6 x G-LLA-F3).

Locus pair	<u>Ab+aB</u> <u>aB</u> <u>Ab</u>	<u>Ab</u> <u>ab</u>	<u>AB</u> <u>aB</u>	<u>AB</u> <u>Ab</u>	<u>aB</u> <u>ab</u>	<u>aB</u> <u>aB</u>	<u>Ab</u> <u>Ab</u>	<u>AB</u> <u>AB</u>	<u>ab*</u> <u>ab</u>	N	χ^2 #	P
<u>Fan</u> - <u>Ap</u>	56	23	29	30	18	14	19	9	19	217	10.10	0.20
<u>Fan</u> - <u>Idh1</u>	43	33	27	23	26	20	14	13	13	212	7.45	0.30
<u>Fas</u> - <u>Ap</u>	56	25	27	32	24	11	16	10	16	217	3.76	0.70
<u>Fas</u> - <u>Idh1</u>	51	34	24	21	20	12	13	24	13	212	14.02	0.05

* 'A' refers to the Fan or Fas alleles, 'a' refers to the fan or fas^a alleles. 'B' refers to the Ap-c or Idh1-a alleles, 'b' refers to the Ap-b or Idh1-b alleles.

Chi-square goodness-of-fit test to a 4:2:2:2:2:1:1:1:1 ratio.

Table 2. The F2 segregation ratios of alleles from pairs of loci in the progeny of the cross (N78-2245 x C1640).

Locus pair	<u>Aa</u> <u>B</u>	<u>AA</u> <u>B</u>	<u>aa</u> <u>B</u>	<u>Aa</u> <u>bb</u>	<u>AA</u> <u>bb</u>	<u>aa*</u> <u>bb</u>	N	χ^2 #	P
<u>Fan</u> - <u>Aco4</u>	77	34	32	28	16	13	200	2.55	0.70

* 'A' refers to the Fan allele, 'a' refers to the fan allele. 'B' refers to the Aco4-a allele, 'b' refers to the Aco4-b allele.

Chi-square goodness-of-fit test to a 6:3:3:2:1:1 ratio.

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 1) Rmd and E3 linkage.

Buzzell and Palmer (1987) reported a possible linkage between Rmd/rmd and E3/e3. Rmd gives resistance to powdery mildew caused by Microsphaera diffusa Cke & Pk (Buzzell and Haas, 1978). E3 gives a delayed flowering response to natural daylength extended to 20 h with cool white fluorescent light (Buzzell, 1971).

From the cross of 'Minsoy' (Rmd e3) x 'Hark' (rmd E3), F3 progeny of F2 plants were rated in a greenhouse for reaction to powdery mildew and for response to daylength extended to 20 h with cool white fluorescent light. Results for 721 F2 plants, including those previously reported (Buzzell and Palmer, 1987), are as follows: 412 Rmd E3 : 140 Rmd e3 : 139 rmd E3 : 30 rmd e3. Using the procedure of Immer and Henderson (1943) an estimate of recombination of 43.6 ± 3.1 S.E. was obtained. The results indicate that the Rmd and E3 loci are loosely linked; neither locus has been mapped to a linkage group.

In other work involving E3, a cross was made between the 'Harosoy' isolines L71-111 (E3 dtl E1 T) and L71-802 (e3 Dtl E1 T) from Illinois. An F2 population was grown in the field in 1987; 174 plants were rated as having indeterminate stem type and 52 as being determinate. On this E1 background there was little indication of the intermediate semi-determinate class that was observed by Bernard (1972). The 52 determinate plants were saved and F3 progeny were grown in 1988; of these, 49 were non-segregating for determinate plant type. The observed 177 indeterminate : 49 determinate gave a good fit ($P=0.30-0.20$) for a 3:1 ratio expected for the segregation of Dtl/dtl. The determinate F2 plants were progeny-tested during the winter of 1987-88 for response to daylength extended to 20h with cool white fluorescent light. There were 28 E3E3 : 19 E3e3 : 2 e3e3, which does not fit ($P<0.001$) the 1:2:1 ratio expected for segregation of E3/e3 independent of Dtl/dtl. These results indicate that E3 is linked with dtl. The dtl gene is linked to L1 ($R=39.4 \pm 1.8$) in linkage group 5 (Weiss, 1970). Tests are being done to map E3 and Rmd in relation to dtl and L1.

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1. An osmotin-like protein from cultured soybean cells.

The discovery of osmotins in plant cell cultures (Singh et al., 1987) brought molecular biology into the study on mechanism of plant osmotic regulation. Here we report results on an osmotin-like soybean protein that was extracted with water or Tris HCl buffer in order to make its further purification easier.

Materials and methods: Soybean cell suspension (Glycine max Merr. cv. Mandarin) was normally subcultured in B5 medium (Gamborg et al., 1968). NaCl-adapted cells were obtained by transferring normal cells into a medium added 0.5% NaCl. In order to test whether the influence of NaCl on the cell protein composition is special for the salt, another cell line was subcultured in B5 medium, supplemented with 6% polyethyleneglycol (PEG).

Cells were harvested as described by Singh et al. (1985). Water- and Tris HCl buffer- (250 mM, pH 6.8) soluble proteins were extracted in a sequential way. Porosity gradient polyacrylamide gel electrophoresis (Poro-PAGE) 4-30% in Tris HCl pH 8.8 or 6-24% in Tris Borate buffer pH 8.9 was used in separating native cell proteins. SDS Poro-PAGE in Tris HCl buffer was used for separation of SDS-denatured proteins. Uncoiling for SDS-PAGE, native protein samples were heated in a boiling bath for 5 min with lysis buffer (Tris HCl 250 mM, pH 6.8, 1% SDS, 1% mercaptoethanol, 10% glycerol) before application to electrophoresis.

Results and discussion: Patterns of water- and buffer-soluble proteins from soybean cells separated in Tris buffered gel showed a new protein with fast mobility in case the cells were grown in the medium with 0.5% NaCl. Some other proteins enhanced while some bands became faint after cells were treated with NaCl. In Tris Borate buffered gel, similar protein patterns were obtained except the new protein consisted of two bands. The separation of uncoiled proteins by SDS Poro-PAGE confirmed the results with native proteins. The major protein component accumulated in salt-treated cells has an apparent molecular weight of about 17 kilodaltons in both water- and buffer-soluble proteins. Since the new protein also occurred when soybean cells were transferred into a medium supplemented with 6% PEG, it may be similar to osmotins from tobacco and tomato cells (Singh et al., 1985) except for its lower molecular weight.

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1) Response of soybean to low temperature during germination.

Littlejohns and Tanner (1976) reported that when emergence experiments were conducted on 32 soybean cultivars at 10 C, 20 C, and 30 C, the results of emergence experiment at 10 C (but not at 20 C and 30 C) permitted classification of cultivars into cold-tolerant, intermediate, and intolerant groupings. Szymer and Szczepanska (1982) observed that strain S-78/TE showed the highest seed vigor in laboratory tests and the best emergence counts in field experiments after they studied cold tolerance of soybean seeds during germination with 25 soybean genotypes over three years. Zhang and Zhang (1988) reported that soybean plants grew slowly, prolonged growth period, decreased yield under treatment of low temperature, and the period of flower bud differentiation with most sensitivity to low temperature was the key stage of cool injury of soybean.

The aim of this experiment was to provide some cold-tolerant genotypes of soybean during germination to related producers and research institutions for direct and indirect uses, and to seek relationships between seed characters and tolerance to cold and the correlations between dry seed chemical compositions and cold tolerance.

Materials and methods: A total of 1,910 cultivars (or strains) from the northeast of China were tested in 1987 and 1988. The seeds of each cultivar were sterilized by soaking in 5% sodium hypochlorite and rinsing in sterilized distilled water and were placed on filter papers in sterilized plastic or glass dishes which were partly filled with sterilized distilled water. Then the dishes with seeds were orderly replaced in controlled phytotrons at 6 C, without light, for 25 days. Water in the dishes was changed once every three days. The same seeds were incubated at 25 C, with three replications as a control. Germination percentage was recorded and relative germination ratio (RGR), which was considered as a cold-tolerant criterion, was calculated according to the following formula

Relative Germination Ratio =

Percentage of Germination at 6 C

Percentage of Germination at 25 C

The ranks of tolerance to cold were given as follows:

- 1) RGR that was above 85% was highly resistant to cold (HR).
- 2) RGR from 50% to 84.9% was scored as mid-resistant to cold (MR).
- 3) RGR that was 50% below was graded as susceptible to cold (S).

Seed characters were measured and dry seed chemical compositions were analyzed in advance by the scientists and technicians working in

agriculture sciences academies or institutes in Heilongjiang, Jilin, and Liaoning provinces. We are grateful to them!

Results and discussion: Germination Percentage Germinated at 6 C and Cold Tolerance -- The germination percentages from different cultivars (or strains) varied greatly, ranging from 0 to 100%, which showed different resistances to cold for soybeans. Thirty cultivars (3%) germinated at 6 C quickly with their germination percentages up to 50% after 8 days, in 1987 (Table 1). The rates of cultivars from Heilongjiang germinated at 6 C up to 50%, after 8 and 11 days, were higher than those of both Jilin and Liaoning, at 6 C in 1987 (Table 1). The rate of cultivars (11.9%) from Jilin germinated at 6 C, up to 85% after 14 days, was the highest of those from the three provinces in 1987, and the rate of cultivars (42.1%) from Jilin germinated at 6 C, up to 85% after 10 days, also was the highest of those from the three provinces in 1988 (Table 2). There were 93 (9.4%) cultivars that germinated at 6 C up to 85% after 14 days in 1987, and 247 (26.7%) after 10 days in 1988 (Table 2).

Relative Germination Ratio and Cold Tolerance -- When RGR was considered as a cold-tolerant criterion, 92 (9.3%) and 266 (28.8%) cultivars, which were classified as highly resistant to cold, were separately attained in 1987 and 1988 (Table 3). This experiment proved that RGR used as a criterion of cold tolerance was more accurate with an extra control germination test at 25 C, compared with percentage of germination treated at low temperature.

Table 1. Number of cultivars that germinated up to 50% at 6 C for different days in 1987.

Province	No. of cultivars	8 days		11 days		14 days		19 days		25 days	
		No.	%	No.	%	No.	%	No.	%	No.	%
Heilongjiang	305	12	3.9	100	32.8	185	60.7	237	77.7	278	91.1
Jilin	360	13	3.6	91	24.3	171	47.5	252	70.0	291	80.0
Liaoning	321	5	1.5	67	20.9	165	51.4	249	77.6	281	87.5
TOTAL	986	30	3.0	258	26.2	521	52.8	738	74.8	850	86.2

Relationship Between Seed Character and Tolerance to Cold -- Soybean cultivars with black seed coats were the highest in tolerance to cold of all those in 1987 and 1988 with a cold tolerant order: Black>Saddle and Tiger's Spot or Brown>Green>Yellow (Table 4). Tully et al. (1981) reported that soybeans with black-pigmented seed coats imbibed water more slowly than nonpigmented seed coat soybeans and showed less vigor loss following imbibition at 6 C, and exhibited no cellular damage as determined with TTC stain. Soybeans with deep brown hilum showed higher tolerance to cold, compared with those with yellow hilum, light brown hilum, and brown hilum (Table 4). Soybean cultivars with kidney seeds exhibited highest resistance to cold of all (Table 4). Little difference in cold tolerance was discovered between yellow cotyledon and green

cotyledon (Table 4.) The order of tolerance to cold among soybeans with different seed coat lustres was found as follows: Non>Slight>Strong (Table 4).

Table 2. Number of cultivars germinated at 6C up to 85% in 1987 and 1988.

Province	-----1987-----				-----1988*-----			
	No. of cultivars	85% above			No. of cultivars	85% above		
		No.	%			No.	%	
Heilongjiang	305	22	7.2		308	53	17.2	
Jilin	360	43	11.9		428	180	42.1	
Liaoning	321	28	8.7		188	14	7.4	
TOTAL	986	93	9.4		924	247	26.7	

* Because the seventh day treated at 6 C happened to cut power for 5 hours and the temperature in the phytotron rose in 1988, the germination percentages increased more rapidly than those in 1987.

Table 3. Number and percentage of different types of tolerance to cold in soybean

Province	-----1987-----						-----1988-----					
	HR		MR		S		HR		MR		S	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Heilongjiang	22	7.2	163	53.4	120	39.3	55	17.9	173	56.1	80	26.0
Jilin	43	11.9	128	35.6	189	52.5	193	45.1	222	51.9	13	3.0
Liaoning	24	8.7	137	42.8	156	48.6	18	9.6	139	73.9	31	16.5
TOTAL	92	9.3	428	43.4	465	47.2	266	28.8	534	57.8	124	13.4

Seed Size and Chemical Composition Correlated with Cold Tolerance --
The weight of 100 seeds correlated negatively with cold tolerance up to 1% significance level in 1987 and 1988 (Table 5). Ina et al. (1978) pointed out that soybeans germinated at 20 C, 25 C, 30 C, 35 C with the same order of germination percentages and potentials as follows, with different water treatments: Small>Middle>Large seed size. Dry seed fat percentage and proline percentage also correlated negatively and significantly with tolerance to cold (Table 5). Linoleic/oleic (L/O) acid ratios correlated positively and markedly with cold tolerance, up to 1% significance level (Table 5). A little correlation was revealed between protein percentage and cold tolerance (Table 5). Lesaint-Onervel (1969) presented that soluble proline percentage in plants and cold-acclimated plants correlated positively with organ's tolerance to cold. However, the result given above by us was different from that. That would keep us going on with further research. Bartkowski et al. (1977) found that low correlation existed between unsaturated/saturated (U/S) fatty acid ratios of total seed lipid and laboratory germination percentages and that seedling

Table 4. Relationship between seed character and tolerance to cold in soybean

Item	-----1987-----		-----1988-----	
	No. of cultivars	Mean of RGR	No. of cultivars	Mean of RGR
Yellow	784	47.4	710	66.0
Green	52	54.2	69	67.5
Brown	37	56.7	64	74.3
Black	49	66.0	66	82.3
Saddle & Tiger's Spot	13	59.6	9	70.8
Yellow	168	48.2	128	71.5
Light brown	163	45.6	189	70.3
Brown	369	48.5	339	62.0
Deep brown	100	52.3	133	72.9
Blue	26	43.0	19	78.1
Black	105	59.1	110	70.1
Circular	279	43.5	339	64.2
Oblate	16	57.3	9	51.6
Ellipse	431	49.6	413	67.8
Flat ellipse	99	58.2	49	71.5
Long ellipse	82	46.9	61	73.4
Kidney	27	78.3	48	88.1
Yellow	120	52.4	149	68.5
Green	35	52.9	31	68.7
Non	77	51.2	154	79.3
Slight	190	47.2	185	69.9
Strong	170	42.5	478	65.6

emergence under field conditions with low soil temperatures correlated strongly with U/S fatty acid ratios of total seed and polar lipids in Pima cotton. Zhao and Hu (1986) showed that the higher L/O acid ratios contained in dry embryos in maize, the stronger seed germination ability exhibited at low temperature, which corresponded with our results. Unander et al. (1986) reported that oil concentration showed a small significant positive correlation with cold germination that was contrary to our results.

Table 5. Seed size and chemical composition correlated with cold tolerance in soybean

Item	-----1987-----		-----1988-----	
	Df	r	Df	r
Protein	500	0.037	867	-0.055
Fat (%)	500	-0.110*	866	-0.109**
Proline (%)	500	-0.121**	814	-0.092**
L/O	500	0.205**	500	0.227**
100-seed wt.	500	-0.184**	916	-0.242**

* Significant at 5% level.

** Significant at 1% level

Conclusion: This seed germination test at 6 C indicated generally that soybean cultivars with small and kidney-shaped seeds, black seed coats, deep brown hila, non-luster of seed coats, and with low fat percentage, low proline percentage, and high linoleic/oleic acid ratios in dry seeds showed higher tolerance to cold.

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1) Induced protein content mutation in soybean.

The human demand for soybeans is primarily for its protein and oil content. In recent years, accounts have increased in the world. It is important that breeders provide more productive varieties; also, they should culture varieties with high protein percentage, in the future.

In the present study, an attempt has been made to demonstrate mutagenicity of ethyl methanesulphonate (EMS) as well as sodium azide (NaN_3) on soybean, with special reference to the effect of concentration on inducing protein content mutation. The following purpose was to find the breeding method for increasing protein content of soybean seeds, and to provide materials that have a high percentage of protein, as well as good agronomic characteristics.

Materials and methods: Dry, fully developed seeds of stable line LF81-837, with about 10% moisture content, presoaked 12 h at 16.5 C, were treated with 0.2%, or 0.4% solution of EMS at pH 7, and 0.001, 0.002, 0.005 M of NaN_3 at pH 3. The treatments were carried out for 2 h at 26 C. Then the seeds that the surface had been dried were sown in the field along with untreated control seeds (soaked by water pH 7 lasting same time as treated seeds).

M1 plants were harvested individually in 1985. The seeds per M1 plant were divided into two parts (each 10-15 seeds). One of them was used for plant-to-row progenies for M2 generation. The other part was maintained for M2 population in 1987. The seeds of M2 and M3 generations with the control were planted in the field, harvested and measured for protein content in family per treatment, in 1987.

Results and discussion: Field plant survival in the M1 generation treated with 0.005 M sodium azide solution reduced by 49.9%, compared with the control. In the other four populations, however, the plant survival was not significantly different from the control.

Overall means and variance for protein, based on families of M2, and M3, and control, were slightly changed, but with no significant difference, as shown in Table 1. The means of protein derived from mutagen-treated plants were not significantly different, but there was a wide range of genetic variability of protein content in treated populations. The mutation of protein in soybean seeds exhibited toward the positive, also toward the negative direction. It was worth noticing

that the protein percentage of a quantity of families exceed the maximum value of the control, which we designated as Exceeding Maximum Value of Parent (EMP). A summary of the experiment in which the EMP frequency has been investigated is shown in Table 2 and Figure 1.

Table 1. Plant survival (%) in M1, means (\pm SE) of protein and its heritability for M2 and M3 populations, based on family.

Treatment	Field plant survival (to control) in M1	Mean (\pm SE) of protein content		Heritability for protein	
		M2	M3	M2	M3
Control	100.0	44.9 \pm 0.82	45.2 \pm 0.57	93.7	79.1
EMS 0.2%	110.6	44.9 \pm 0.70	45.4 \pm 0.62	87.5	87.2
0.4%	93.6	45.0 \pm 0.97	45.8 \pm 0.68	92.9	83.4
NaN ₃ 0.001M	117.0	45.0 \pm 0.65	44.7 \pm 0.62	94.0	87.2
0.003M	121.3	45.3 \pm 0.89	45.6 \pm 0.90	91.9	88.9
0.005M	51.1	45.3 \pm 0.64	45.6 \pm 0.66	78.2	76.3

Table 2. EMP frequency and range of protein content in M2 and M3 generations

Treatment	Range of protein content		EMP frequency (% of protein content)	
	M2	M3	M2	M3
Control	42.4-45.7	44.1-46.4	-	-
EMS 0.2%	43.2-46.6	44.1-46.5	3.62	0.51
0.4%	42.8-45.6	44.3-47.1	0.52	2.59
NaN ₃ 0.001M	43.1-47.1	43.6-46.0	1.55	0
0.003M	43.1-46.4	44.1-46.9	1.55	1.55
0.005M	43.6-46.1	44.3-47.0	1.10	0.52

It can be seen that every population treated had a greater frequency of the EMP than the control for the protein content of soybean in the M2 and M3 generations. Although the EMP frequencies were not stable in the M2, sometimes they may change in the M3 generation. According to the EMP frequency and heritability, the study indicated that ethyl methanesulphonate and sodium azide were effective, economical mutagens for enhancing protein content in soybean seeds. The mutations in protein content induced by them could be maintained in the following generations.

Breeders may select high protein percentage lines of soybean, and develop the variety from progenies treated with these two mutagens. Safe concentrations were 0.2-0.4% EMS and 0.003-0.005M NaN_3 solutions. The more appropriate concentrations suggested were 0.4% EMS, and 0.003 M NaN_3 for inducing high protein content in soybean seeds. Of course, the selection for this should be early generation, for example, in the M3.

In this study, measuring for protein content was carried out in the M3 generation, rather than the M2 family, generally, because of physiological damage still remaining in M2 progenies of various treatments with EMS or NaN_3 . There probably were some plants in the families with reduced numbers of pods, seed set per plant and fertility percentage, any of which might affect frequency distribution of protein content.

The efficiency of the mutagens is not only dependent upon concentration, but also on other variables such as pre-soaking time, duration of treatment, pH of mutagen solution, even the temperature during treatment, and so on. We have a few references about it, and intend to deal with that in future programs.

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1) Periodical variation and geographical distribution of protein and oil content of soybean varieties in Heilongjiang Province of China.

In Heilongjiang Province, the protein content of soybean cultivars released in 1960's and 70's was apparently lower than that of the cultivars released in the 1950's; the oil content increased with the passage of time, but the change was not great. So the total content of oil and protein of the soybean cultivars released in the 1960's and 70's was remarkably lower than that of the cultivars released in the 1950's. The cultivars released in the 1980's have a lower oil content and a higher protein content in comparison with the cultivars released in the 1960's and 70's, and their total content of oil and protein is higher than that of the cultivars released in the 60's and 70's and near to that of the cultivars released in the 1950's (Table 1). In the 60's and 70's, soybean breeders put emphasis mainly on high yields and ignored chemical quality. Because seed yields of soybeans have a positive correlation with oil content and a negative correlation with protein content, the oil content of the cultivars released in the 60's and 70's slightly increased and their protein content decreased, then the total content of oil and protein also decreased. In the 1980's, soybean breeders began to pay attention to chemical quality, so the chemical quality of cultivars released in the 80's was slightly improved in comparison with the cultivars released in the 60's and 70's, but had not reached the level of the 50's. From all results above, we find that both intentional and unintentional selections on chemical quality may result in changes of chemical quality. Intentional selection results in changes toward definite direction, and unintentional selection results in fitful changes.

Chemical composition of different variety types are shown in Table 2. The protein content of bred cultivars (released from 1950 to 1986) is lower than that of land races, but their oil and (oil+protein) contents are higher than those of land races. The oil content of cultivars released in the 1980's is higher than that of land races, but almost equal to that of bred cultivars. The protein and (oil+protein) contents of cultivars released in the 1980's are higher than those of both land races and bred cultivars. Generally speaking, in comparison with land races, the chemical quality of bred cultivars is not improved; the chemical quality of cultivars released in the 1980's is slightly improved. Therefore, the potential to improve the chemical quality of soybeans is great.

As to the geographical distribution of oil and protein contents, the land races (or local varieties) in certain regions of Heilongjiang Prefecture and Songhua Prefecture have a higher protein content; the land races in Suihua, Nenjiang, and Hejiang prefectures have a higher oil content. The mechanism of the distribution remains to be studied.

Table 1. Comparison of chemical composition of soybean cultivars released in different times.

Times	Number of cultivars	Oil* (%)	Protein* (%)	Protein + Oil (%)
50's	12	20.39	39.99	60.38
60's	20	20.98	37.75	58.73
70's	24	20.88	37.51	58.39
80's	26	20.79	39.42	60.21
Means	82	20.80	38.54	59.34

*Content on dry matter basis.

Table 2. Comparison of chemical composition of different soybean variety types.

Type of variety	Number of varieties	Oil* (%)	Protein* (%)	Protein + Oil (%)
Land races	147	19.35	39.22	58.57
Bred cultivars	82	20.80	38.54	59.34
Cultivars released in 80's	26	20.79	39.42	60.21

*Content on dry matter basis.

Table 3. Geographical distribution of chemical composition of soybean land races.

Prefecture or city	Number of varieties	Oil* (%)	Protein* (%)	Protein + Oil (%)
Harbin	21	19.05	39.10	58.15
Hejiang	10	19.47	38.41	57.88
Songhuajiang	27	19.11	39.89	59.00
Nunjiang	18	19.67	39.35	59.02
Mudanjiang	5	19.18	39.13	58.31
Suihua	31	19.86	38.46	58.32
Heihe	26	19.36	39.57	58.93

*Content on dry matter basis.

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1) Ecological types of chemical composition of *G. soja* and their eco-geographical distribution.

There is a high content of protein in Glycine soja. It can be an important source of high protein for a soybean breeding program, but the protein content is different in different regions, as is the content of oil. Obviously, there are different ecological types of chemical composition in G. soja. A project for breeding soybeans to improve the chemical content can benefit from a study of ecological types of G. soja and their eco-geographical distribution.

Ecological Types of Chemical Composition of *G. soja*: Five hundred and forty four samples of G. soja, with 1.00-2.99 g/100 seed, collected from each county of Heilongjiang province (the most important province of soybean production in China) were studied for protein and oil content. The results showed that there is a significant difference in protein and oil content in the samples of G. soja from different ecological regions; i.e., there are different ecological types of protein and oil content in G. soja. On the basis of specific expression of protein and oil under given eco-environments, G. soja can be divided into three ecological types. They are: high protein type, high oil type, and high oil and protein type. Also, there are ecological regions for each eco-type.

Factors Affecting Development and Distribution of Chemical Composition Eco-types: 1. Geographical factors -- The important geographical factors affecting ecological types are latitude and elevation. There is a negative correlation between latitude and protein content, also between elevation and protein; there is a positive correlation between latitude and oil, and between elevation and oil (Tables 1 and 2).

Table 1. Protein and oil content of G. soja in different latitudes

Latitude	44	45	46	47	48	49	50	51	Ave.	r
Protein	48.90	47.58	47.44	47.17	45.19	45.47	44.69	47.1	-0.594	
Oil	7.97	8.14	7.80	7.99	7.95	8.23	9.71	8.07	0.370	

Data in Table 2 show that there is not a significant correlation between protein and oil content at 46 - 48 N, and the correlation between oil and elevation is different from other latitude regions. Therefore, there is the possibility of both higher protein and oil in this region.

Table 2. Correlation between elevation and protein/oil content in different latitudes

Latitude	44	45	46	47	48	49	50	51	Ave.
Protein	-0.66**	-0.30*	-0.16	-0.58**	-0.07	-0.57**	-1.0**	-0.35**	
Oil	0.63**	0.15	0.14	0.28*	0.68**	0.16	0.51**	0.27*	
Between									
Protein and oil	-0.91**	-0.58**	0.04	0.04	-0.70**	-0.91**	-0.50**	-0.44**	

When the effect of elevation is not disregarded, there is some difference between protein and oil content in steps of elevation with 50 meters, but it is not significant. Then in regions with high latitude and elevation, protein content is significantly lower than in regions with low latitude and elevation. When the step of elevation is 100 meters, protein and oil content show regular variation as the elevation changes. With an increase in elevation, there is a decrease in protein content, and an increase in oil content (Table 3).

Table 3. Effect of elevation on protein and oil content

Elevation	0	50	100	150	200	250	300	350	400	Ave.
Protein	45.77	48.23	47.20	47.27	46.94	47.04	45.07	42.29		47.10
Oil	6.72	8.18	7.89	8.06	7.93	8.66	8.21	10.14		8.07

Elevation	0	100	200	300	400	Ave.
Protein	47.92	47.24	46.99	44.14		47.10
Oil	8.00	7.98	8.14	8.85		8.07

2. Meteorological factors: The important meteorological factors affecting development and distribution of the chemical composition eco-types of *G. soja* are temperature, hours of sunshine, and rainfall. The most important factor is temperature. From the first 10 days of July to the last 10 days of September, correlation coefficients between temperature and protein/oil content are more than 0.50 with significance. Higher temperature is favorable to accumulation of protein (positive correlation); lower temperature is favorable to accumulation of oil (negative correlation). In general, during the period from flowering to maturity, the correlation between protein content and average temperature of each Solar Term is going to strengthen gradually. The absolute value

of the negative correlation between oil and average temperature of each Solar Term is somewhat lower than the preceding term (Table 4). The stages affecting protein or oil most strongly are different. This shows that the greatest effect of temperature on accumulation of oil is in its early stage; but that for protein is in the later stage, and in the last stage this effect weakens.

Table 4. Correlation between protein/oil content and average temperature of each Chinese Solar Term

Solar Term	Summer solstice June 21 -	Slight heat July 5 -	Great heat July 23 -	Beginning of autumn Aug 5 -	Limit of heat Aug 23 -	White dew Sept 5 -	Autumnal equinox Sept 23
Protein	0.308*	0.319*	0.432**	0.506**	0.519**	0.508**	
Oil	-0.375**	-0.306*	-0.312*	-0.297*	-0.327*	-0.296*	

There is only a period, from Great Heat (July 23) to Beginning of Autumn (Aug. 22), in which rainfall affects protein content significantly. Correlation coefficient is 0.295*. The oil content is positively related to rainfall from Summer Solstice (June 21) to Slight Heat (July 22), and negatively related to rainfall from Slight Heat (July 5) to Great Heat (Aug. 4), not significantly related to other periods for rainfall. Here, we can find that the period in which oil content is affected by rainfall is earlier than that of protein (Table 5).

Table 5. Relation between protein/oil and rainfall of each term

Solar Term	Summer solstice June 21 -	Slight heat July 5 -	Great heat July 23 -	Beginning of autumn Aug 5 -	Limit of heat Aug 23 -	White dew Sept 5 -	Autumnal equinox Sept 23
Protein	-0.15	-0.08	0.30*	0.18	0.02		-0.17
Oil	0.26*	-0.42**	-0.01	-0.03	0.08		0.12

In the first phase of reproduction growing stage of G. soja, there is a negative correlation between protein content and hours of sunshine. In the last phase, it is a positive correlation. But most of the correlations in the first phase are significant, and most of those for the last phase are not significant. Oil content is related to sunshine negatively. In the region with longer hours of sunshine, there is lower oil content in G.

soja seeds. In apposition to temperature and rainfall, the stage in which the content is significantly affected by hours of sunshine is earlier for protein than that for oil (Table 6).

Table 6. Correlation between protein/oil and hours of sunshine

Period of ten days#	June			July			September			Average		
	F	S	L	F	S	L	F	S	L	June	July	Sept.
Protein	0.07	-0.18		-0.26*			0.16		0.44**		-0.17	
		-0.25*		-0.32**		0.21		0.22		-0.11		0.29
Oil	-0.17	-0.12		-0.01			-0.38**		-0.29*		-0.12	
		0.09		-0.03		-0.34**		-0.39**		-0.17		-0.36**

F, S, and L = first, second, and last ten days of a month.

According to the relationship between protein and oil content and eco-environment and their eco-geographical distribution, Heilongjiang can be divided into four eco-geographical regions, and seven subregions, for protein and oil ecotype distribution of G. soja:

I. High content ecotype region of protein and oil. This is located in the southeast part of Heilongjiang. Here the temperature is not too high and not too low, spring soil is not too dry nor too damp, summer and fall has not too much rainfall. An ecotype with high content of protein and oil can be found in this region.

II. High protein region, including subregions II-1 and II-2. II-1 is located in the northeast of the province. Here it is lukewarm, humid, and there is enough rainfall. But there is a somewhat waterlogged lowland here. II-2 is located in the southern part of the province. Here is the best warm part of the province, with light, dry weather. Spring is somewhat drier and summer is subhumid. An ecotype of G. soja with high protein has developed here. Protein content in this region is higher than the average content of the whole province.

III. High oil ecotype region with three subregions. Subregion III-1 is located in the northern part of the province, a mountainous area. Here it is cold and humid. Sometimes there will be a slight dryness in spring. Summer is subhumid. III-2 is the northwest part of the province. It is cool and subdry. Spring is dry; summer is humid. III-3 is a mountainous area, too. It is located in the middle south of the province. Here it is cool and humid. Spring is slightly dry; summer is humid. An ecotype of G. soja with high oil content has developed in this region; its oil content is higher than the average of the whole province.

IV. Low content ecotype region. This region is located in the western part of the province. It is warm and dry. Spring drought is

frequently serious. Summer is subhumid. An ecotype of G. soja with low content of protein and oil developed here...the lowest in the province.

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1) Relationship between germinability after accelerated aging, laboratory germination and seed size in soybean.

It is essential to develop high yielding soybean varieties having better seed longevity. Present investigation deals with the correlation coefficients between germinability after accelerated aging, laboratory germination and seed size in 331 germplasm lines of soybean to ascertain the utility of accelerating aging stress test in screening for seed germinability and to test if seed size could be a simple criterion for seed germinability.

Materials and methods: A total of 331 lines were evaluated under this investigation.

The accelerated aging originally proposed by Byrd and Delouche (1971) involved keeping seeds at 42 C and 100% relative humidity for 48 hours, followed by laboratory germination test. In this investigation, this method was slightly modified in order to get-clear cut differences between poor and better germinating seeds. In this modified test, the seeds (50 seeds/line) were aged at 40 C and 100% relative humidity for 96 hours. For germination test seeds were kept in petri dishes on moist paper towel/filter paper at 30 C in incubator. After 3-4 days seeds were classified into three categories, viz., germinated seeds (well developed primary roots and hypocotyl), hard seeds and dead seeds. The germinated seeds had longer radicle and hypocotyl (about 3-4 cm). Seeds which did not imbibe water were counted as hard seeds.

The electrical conductivity of seed leachate was also determined with the help of conductivity meter and expressed as mmhos/cm at 25 C (Matthews and Bradnock, 1968) on another set consisting of 6 parental lines and 15 Fl's. Seed size was expressed as 100-seed weight (g).

Results and discussion: Correlation coefficients between germinability after accelerated aging, laboratory germination, and seed size are given in Table 1.

The germinability after accelerated aging stress was positively correlated with laboratory germination. Seed size was negatively correlated with germinability after accelerated aging and laboratory germination both. The correlation coefficient between germinability after accelerated aging (including hard seeds) and EC of seed leachate was nonsignificant and positive (0.214), it was negative and significant (-0.466) at the 5% level of probability, when hard seeds were excluded.

Germination percentage was negatively correlated with 100-seed weight (Table 1, $r=-0.277$, including hard seeds; $r=-0.333$, excluding hard seeds).

Table 1. Correlation coefficients between germinability after accelerated aging, laboratory germination and seed size in 331 germplasm lines of soybean.

Character pair	Correlation coefficients including hard seeds	Correlation coefficients excluding hard seeds
Germinability after accelerated aging test and laboratory germination	0.377**	0.395**
Seed size and germinability after accelerated aging test	-0.186**	-0.253**
Seed size and laboratory germination	-0.227*	-0.333**
Germinability after accelerated aging test and electrical conductivity (in 6 parents + 15 Fl's)	0.214	-0.466

* Significant at 5% probability level.

** Significant at 1% probability level.

Negative association between germination and seed size has been reported by Edwards and Hartwig (1971), Paschal and Ellis (1978), Singh et al., (1978), and Rana et al. (1982) in soybean.

It is, therefore, suggested that accelerated aging stress can be successfully utilized in screening of cultivars/breeding lines for germinability. Based on seed size, also, it may be possible to screen cultivars for better germinability. Edwards and Hartwig (1971) found that small seeds were associated with rapid hypocotyl development and better germination in soybean. Nangju (1979) suggested to screen soybean cultivars for resistance to weathering on the basis of seed size and the proportion of smooth and clean seeds. Similarly Wien and Kueneman (1981) reported that some small seeded lines of southeast Asia origin (TGm 737, TGm 685, and TGm 693) maintained more than 50% germinability after 9 months of adverse ambient storage.

In conclusion, we suggest that it is possible to screen soybean lines for germination by accelerated aging stress test and seed size.

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2) Glycine soja - a source of resistance for Bihar hairy caterpillar, Spilosoma (=Diacrisia) obliqua Wallace, in soybean.

Glycine soja L. Sieb. & Zucc. syn. G. formosana Hosokawa was originally introduced at this university from Taiwan Agricultural Research Institute, Taipei, in the early seventies. While screening the 5000 germplasm lines for resistance to disease, G. formosana, along with PI 171443, was shown to be completely free from yellow mosaic (Singh et al., 1974). Since then these are being used as sources of resistance to yellow mosaic virus in our regular soybean breeding program. G. soja is a typical wild-looking soybean, with small, very narrow leaves and prostrate growth habit. It flowers in about 85 days and matures in about 130 days. It has tawny pubescence and purple flowers. It is susceptible to bacterial pustules and rust. It crosses easily with cultivated soybeans. Several yellow mosaic-resistant new breeding lines of soybean, including a very promising entry PK-515, have been developed from crosses involving this wild soybean (Ram et al., 1984) and this process is still continuing.

An additional remarkable feature of G. soja concerning resistance to Bihar hairy caterpillar was noted during rainy season, 1988. Bihar hairy caterpillar is a serious insect for most of the legumes, primarily soybean, during the rainy season in the northern part of the country. The larvae eat the leaf lamina completely and the plant is left virtually with only leaf petioles and pods, and the forced maturity sets in. Depending upon the stage of plant infestation, the yield loss may vary from negligible to 100%. When the infestation takes place during the initial podding stage, the yield loss is almost complete. When the infestation occurs at full pod stage (R4-R6), the seed size is considerably (about 40%) reduced.

During the rainy season 1988, the infestation of this defoliator was very severe. No insecticide could be sprayed effectively due to labor unrest from 19 September to 16 October. During this period, about one third of our breeding block (5 acres), having been sown late (20 July) for multiplication of a few promising lines, was completely defoliated and seed yield was reduced by about 80%. Resultant seeds were small. In this case, damage was severe, as the crop was in R3 to R4 stage. In the remaining block of 10 acres, the defoliation progressed a little late, as the larvae were eating and migrating from one side of the field (5-acre block side) to the other side, i.e., 10-acre block side. Further, this 10-acre block had the crop in an advanced stage (R4 to R6) due to normal time of sowing (29 June to 3 July) during the infestation period. Thus, this block, too, was completely defoliated but seed development was still satisfactory, though substantially reduced in size. In such a situation of epidemic and devastating infestation of the soybean crop in a 15-acre block by this insect, there was virtually no chance of any escape. However, G. soja planted in the hybridization block remained completely free from Bihar hairy caterpillar, while all around there was complete defoliation in other entries. Hundreds of larvae were seen moving across

the rows of this wild-looking soybean. Not only that G. soja was free from this insect, but a breeding line PK-515, having G. soja in its parentage, was only partially damaged and could be spotted easily in the field. Similarly, an F2 generation of a cross having G. soja as one of the parents (Glycine soja x PK-472) showed a few narrow leaf plants having negligible infestation. Therefore, the resistance detected in this line appears to be inherited and as such it could be used as an effective source of resistance to Bihar hairy caterpillar in a soybean breeding program. The basis of the resistance appears to be chemical, as no physical factor (pubescence, etc.) appeared to be involved. The real basis of this resistance and its precise mode of inheritance needs to be investigated.

The use of G. soja in a breeding program could prove to be of further advantage, as Lee and Wang (1988) have suggested introduction of high protein genes from G. soja into the cultivated type.

PK-564, another soybean line (UPSM-534 x S-38) x Bragg also showed moderate resistance. This line is already identified for release in the northern plains. PK-515 (G. formosana x Bragg) x Bragg is in the coordinated initial evaluation trial and may be released in the future, heralding a new era in breeding, and release of soybean varieties having resistance to insect pests, which is one of our important breeding goals.

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1) Development of high yielding, short stature, and early mutant in soybean.

Introduction: In view of the national and international importance of soybean, a mutation breeding program was initiated at AEARC, Tandojam, to develop new varieties of soybean with improved yield and oil content. Preliminary results of these investigations have already been reported (Rajput and Sarwar, 1988). This report describes and discusses the performance of mutants developed from variety 'Improved Pelican'.

Materials and methods: Air dried, homogeneous seed of Improved Pelican (a tall variety with long maturity period, having indeterminate growth habit) was treated with different doses of gamma rays and Neutron fast. In the M2, putative mutants with high yield potential, along with earliness, determinate growth habit and short stature, were selected and confirmed for their breeding behavior during subsequent generations (M3 and M4). Seven mutant lines from the M4 generation showing uniform and stable breeding behavior were evaluated, along with their parents during summer of 1988 (June-September) in a randomized complete block design with four replications. Four rows 4 meters long of each genotype were planted, keeping inter- and intra-row spacing of 45 and 10 cm, respectively. At maturity, 15 plants were selected at random from each genotype and morphological data regarding plant height, branches per plant, days to maturity, pod length, number of grains per pod, seed index, pods per plant, grain yield per plant, grain yield kg/ha and oil content were recorded. Maturity period was recorded at 50% physiological maturity. Oil content was determined, using Soxhlet apparatus. Grain yield data were subjected to analysis of variance and means were compared, applying DMR test (Steel and Torrie, 1960).

Results and discussion: Behavior of various morphological attributes depicted in Table 1 indicates that the maturity period of mutant lines was reduced by 4 to 10 days, compared with the parent. Maximum reduction of 10 days in maturity was for mutant line IP 2/85 (108 days). The early mutant IP 2/85 showed determinate growth habit, accompanied by short stature (83.5 cm). Plant height in mutant lines ranged from 83.5 to 126.9 cm, compared with 123.36 cm of the parent. Mutant line IP 2/85 also exhibited improvement in branches per plant (4.16) followed by mutant IP 12/85 (3.9) and PI 2/1/85 (3.75). Seed index (100-grain weight) showed a little improvement and ranged between 10.39 and 11.55 g. The main improvement was observed in the number of pods per plant. Pods per plant ranged from 85 to 156.5 in the mutant lines, whereas the parent had 101 pod/plant. Mutant IP 2/85 bore the highest number of pods (156.5).

Significant differences were observed for grain yield among the mutant lines and the parent. Maximum grain yield was produced by IP 2/85 (3124 kg/ha) followed by IP 6/85 (3022 kg/ha), IP 20/1/85 (2865 kg/ha) and IP 4/85 (2779 kg/ha). Yield is a multifaceted characteristic and is the ultimate result of different yield components such as pods per plant, pod length, seeds per pod, and grain size. Hence, any change in these attributes will accordingly affect yield potential.

Oil content of mutants was not much affected, showing a range between 16.62 and 18.25%, compared with 17.87% of the parent. The objective of this investigation to induce short stature mutants with determinate growth habit was achieved to a great extent, along with improvement in grain yield, without unbalancing the oil content. Mutant IP 2/85 gave maximum grain yield and in oil content is at par with the parent. Due to short stature and determinate growth, the mutant is lodging-resistant and better responsive to inputs. Hopefully, after thorough investigation, mutant IP 2/85 will be released as a new improved soybean variety.

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 1) The effect of whitefly (*Bemisia tabaci* Genn.) damage on yield and yield components in double-cropped soybean production.

Cukurova region, located in the south of Turkey, has the largest soybean planting area. In this region, soybean can be grown as a second crop after wheat harvesting (85%). The major insect pest of soybean and cotton in the Cukurova region is the sweet potato whitefly (*Bemisia tabaci* Genn., Aleyrodidae, Homoptera) (Cinar et al., 1985).

Previous studies (Vaishampayan et al., 1980; Ozgur, 1986) indicated that whiteflies lay white-yellow stalked eggs, mostly on the underside of leaves. Larvae are oval and depressed, pale to greenish yellow and 0.5 mm in size. Adults are small insects with yellow body and hialine wings covered with a white powdery wax; it is 1.0-1.4 mm in size.

Injury to soybeans is caused both by larvae and adults sucking sap from leaves. Whiteflies also secrete abundant honeydew on which grows a sooty mold. The black mold interferes with normal photosynthesis processes (Vaishampayan et al., 1980).

Infestation of whiteflies is usually heaviest during the pod-filling period and can cause severe reductions in yield (Ozgur et al., 1986). One hundred and nine soybean varieties were screened between 1976 and 1984 in Cukurova. Some of these varieties were found very resistant and others were very susceptible to whitefly (Arioglu, 1987). Previous studies (Arioglu et al., 1988) indicated that pubescence type and density affected the whitefly infestation in soybean. It is known that whitefly can cause yield reduction in soybean. The purpose of this study was to determine how the whitefly damage affects seed yield and yield components.

Materials and methods: This study was conducted in the experimental area of the university of Cukurova in 1988. The experimental design was a randomized block with three replications. Some 'Clark' isolines, 'Williams 82' and 'Amsoy 71' soybean varieties were used as experimental material. Clark was found very susceptible to whitefly (Arioglu, 1987) and it has 100 days growing period at the double-cropped conditions. Amsoy 71 (resistant) and Williams 82 (susceptible) varieties were used as a standard. The seeds were planted June 20 and were inoculated with *Rhizobium japonicum*. Plot sizes were 2.8 m x 4.0 m = 11.2 square meters; row spacing was 0.7 m. The seeding rate was 30 plants per m row. Irrigation was applied by flood irrigation system 4-5 times. Insecticides were not used for whiteflies over the growing period. Since whiteflies

were very abundant, no artificial infestation was made. The whitefly observation was made on Sept. 7. which is the time of the heaviest whitefly infestation. Ten plants from each plot and three leaves from each plant (lower, medium, and upper parts) were obtained for investigation of the whitefly population. Eggs, larvae, and pupae numbers were counted on the leaves under the microscope (2.85 cm). Seed yield, pod number and seed number per plant, and 100-seed weight were investigated, using INTSOY methods at harvesting.

Results and discussion: Pubescence type and densities of the isolines are given in Table 1.

Table 1. Pubescence type and density of some Clark isolines and soybean varieties.

Clark Isolines and varieties	Pubescence type	Pubescence density* (1-10)
Amsoy 71	Medium long, acute and prostrate	6
L 62-1385	Glabrous	1
L 62-2999	Very long, acute and prostrate	2
L 67-497	Medium long, acute and prostrate	6
L 67-1291	Medium long, acute and prostrate	4
L 63-2435	Medium long, curly and prostrate	6
L 64-314	Short and long, curly and prostrate	7
Williams 82	Medium long, acute and prostrate	4
Clark L-1	Long, acute and prostrate	4
L 75-6648	Short, acute and semi-prostrate	8
L 62-1686	Short, acute and erect	8
L 79-1815	Very short, acute and erect	10

* Pubescence density (1-10): 1=glabrous, 2=sparse, 3-4=semi-sparse, 5-6=semi-dense, 7-8=dense, 9-10=heavily dense.

The pubescence type and densities were found very different from each other isolines. A significant correlation was found between pubescence density and whitefly infestation, but the effect of pubescence density was not a consistent factor. However, pubescence type also has effect on whitefly infestation.

The data belonging to the egg+larvae+pupae numbers, pod number, seed number, 100-seed weight and seed yield are given in Table 2.

Eggs+larvae+pupae numbers of whitefly varied between 7.12 and 99.98 per 2.85 cm leaf area in the tested isolines and varieties. The highest whitefly infestation was observed in the L 79-1815 isoline (Table 2). Therefore, the seed yield was very low in this isoline. Amsoy 71 and L 62-1385 were not affected by whiteflies and the highest seed yields were obtained from these two. Whitefly infestation negatively affected the

Table 2. The average egg+larvae+pupae numbers, pod number, seed number, 100-seed weight, and seed yield data of some Clark isolines and soybean varieties.

Clark isolines & varieties	Whitefly egg+larvae+pupae nos. (2.85 cm)	Pod numbers (N/plant)	Seed numbers (N/plant)	100-seed weight (g)	Seed yield (kg/ha)
Amsoy	7.12	50.20	91.23	17.41	2648.80
K 62-1385	10.01	57.00	97.36	17.99	2447.60
L 63-2999	18.28	42.00	84.47	16.11	2321.43
L 67-1291	24.70	40.97	78.47	13.28	2185.70
L67-497	27.00	56.43	97.33	15.08	1851.20
L 64-314	38.66	39.13	74.57	12.34	1357.13
L 63-2435	44.45	39.33	74.70	14.12	1602.40
Clark L-1	50.89	36.97	78.83	11.82	970.27
L 62-1686	68.44	29.47	57.33	10.46	797.63
L 75-6648	69.41	34.97	68.43	11.76	930.97
Williams 82	73.46	37.17	77.10	10.90	972.27
L 79-1815	99.98	32.60	65.90	10.86	858.13
- - - - -					
CV (%)	18.47	6.87	8.73	7.01	7.85
LSD (%5)	13.87	4.81	11.66	1.62	209.75

seed yield and yield components. The relation between whitefly infestation and seed yield is shown in Figure 1.

A semi-logarithmic relation was found between whitefly infestation and seed yield (Fig.1). The whitefly infestation was heaviest during the pod formation and pod filling period. The soybean plant could not grow normally due to whitefly damage, and it was forced to ripen earlier than the normal maturity. As seen in Table 2, seed yield and yield components were negatively affected by the whitefly damage. Negative correlations were found between whitefly infestation and pod numbers ($r=0.711$), seed numbers ($r=0.659$) and 100-seed weight ($r=0.789$). These are the important yield components in soybean. Positive correlations were found between seed yield and pod numbers ($r=0.764$), seed numbers ($r=0.694$), and 100-seed weight ($r=0.874$). These findings are in agreement with results of Jackobs et al., 1984, Atakisi, 1978, and Arioglu et al., 1986.

In conclusion, significant and negative correlations were found between whitefly infestation and seed yield. Pubescence type and density affects whitefly infestation. Chemical control of the whitefly is very expensive and insecticides are losing their effect rapidly. For this reason, the varieties that are grown in Cukurova have to be resistant to whitefly.

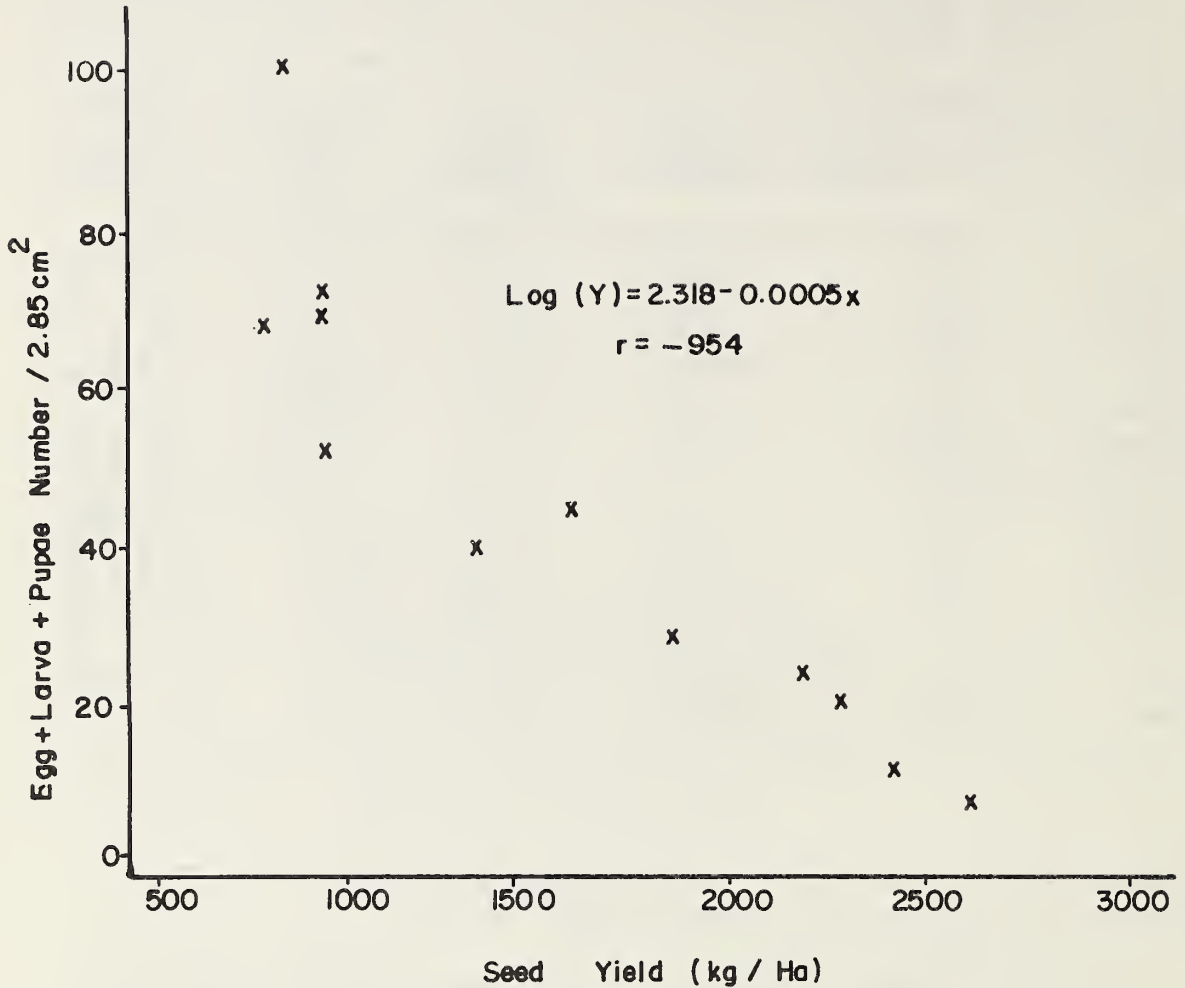


Figure 1. The relation between whitefly infestation and seed yield.

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2) Research on the growing possibilities of some soybean varieties as a main crop in Cukurova, Turkey.

The Cukurova region is located in the south of Turkey and has the most productive land of the country. The main crops are cotton and wheat in this region. Because of suitable soil and climate conditions, it is possible to grow soybean as a main and second crop in Cukurova. In this region, soybean farming is developing very rapidly. While the planting area was 4850 ha in 1981, it reached 90,000 ha in 1987. More than 95% of the total soybean production was obtained from Cukurova (Arioglu, 1988). In this region, soybeans can be grown mainly as a second crop after wheat harvesting. Some of the farmers in Cukurova want to grow a main crop of soybeans instead of cotton, because the cost of cotton production is increasing very much year to year. There is also a marketing problem with cotton. At this time, soybeans are more profitable than cotton as a main crop for the farmers.

Previous studies (Arioglu et al., 1986; Atakisi, 1978; Atakisi et al., 1980) indicated that early varieties belonging to Maturity Groups I, II, III, and IV are grown as a second crop in the Cukurova region. There is about 120-130 days growing period for double-cropped soybean production in Cukurova. But the growing period is about 160-180 days for a main crop of soybean production.

The objective of this study was to determine the high yielding soybean varieties that can to be grown successfully as a main crop in the Cukurova region.

Materials and methods: This experiment was conducted as a main crop in the experimental field of the University of Cukurova, Faculty of Agriculture, in 1987. In this research, 23 soybean varieties were used. The experimental design was a randomized block with four replications.

The climatic data over the growing period in Adana in 1987 are given in Table 1.

Table 1. Climatic data over growing period in Adana, Turkey, in 1987*

Months	Temperature			Relative humidity (%)	Total rainfall (mm)
	Max. C	Min. C	Average C		
April	28.8	6.5	16.1	67	15.8
May	37.9	10.3	20.5	67	38.2
June	37.6	16.2	25.2	65	3.9
July	38.4	18.2	28.2	68	55.8
August	40.3	18.4	28.4	66	48.4
September	39.4	15.7	26.6	59	---
October	36.6	7.4	20.7	54	76.5

*Source: Meteorological Surveys of Adana.

The soil type of the experimental area is sandy loam and soil pH ranges between 7.4 and 7.7. The soil contains a high amount of lime. The soil organic matter is low.

The previous crop was cotton in the experimental area. After cotton harvesting, soil was plowed by a moldboard plow, and seedbed was prepared with a disc harrow at planting time. Fertilizer (200 kg/ha diammonium phosphate) was broadcast at the time of soil preparation. Plot sizes were $2.8 \times 5.0 = 14$ square meters; row spacing was 0.7 m. The soybean seeds were inoculated with *Rhizobium japonicum* and planted by hand April 20. Seeding rate was 25 plants/m row and planting depth was 4-5 cm. The experimental area was furrowed twice during plant development to control weeds; irrigation was applied by flood irrigation system four times. The plots were harvested by hand at different times when the varieties reached maturity and threshed by a stationary plot thresher. Growing period, seed yield, pod number per plant, plant height, 100-seed weight, and the lowest pod height were investigated using INTSOY (1984) methods.

Results and discussion: In this study, growing period, seed yield, pod number, plant height, 100-seed weight, and the lowest pod height were investigated. Data obtained from the research are summarized in Table 2.

It can be seen in Table 2 that the average seed yield per hectare was between 1682.7 and 3735.3 kg. The highest seed yield was obtained from A-4595 and A-3427 varieties. The lowest seed yield was obtained from

Table 2. Growing period, seed yield, pod number, plant height, 100-seed weight, and lowest pod height in soybean varieties grown in the Cukurova region of Turkey.

Varieties	Growing period (days)	Seed yield (kg/ha)	Pod number (no/plant)	Plant height (cm)	100-seed weight (g)	Lowest pod height (cm)
A-4595	139	3735.33	36.30	113.63	15.53	25.20
A-3427	130	3595.33	43.00	92.77	16.56	14.93
A-3127	125	3440.67	52.97	94.17	16.17	14.43
Brich	135	3426.33	41.13	115.53	18.44	15.63
S-3993	135	3340.67	44.63	105.63	15.09	17.46
A-2943	125	3309.67	50.57	80.37	19.49	10.80
A-3966	139	3278.33	46.50	120.80	15.43	21.53
B50-253	135	3233.33	45.63	109.20	18.48	21.33
A-5149	145	3114.33	32.07	108.70	19.18	39.37
L-4106	145	2888.33	39.53	141.23	18.56	31.70
A-4997	140	2869.00	56.53	98.30	18.85	30.23
A-3803	137	2795.00	35.47	94.47	17.12	13.40
RA-403	145	2640.33	47.10	123.97	17.06	21.17
Essex	160	2614.33	55.33	92.77	14.55	19.60
Mitchell 410	145	2473.66	41.93	129.23	18.20	28.30
Mitchell 450	160	2459.33	45.76	141.80	17.87	27.80
Williams 82	125	2235.66	36.83	111.03	16.22	15.60
S-3031	123	2214.67	41.27	100.47	18.86	18.87
A-5474	160	2190.67	46.70	129.10	15.96	36.67
A-5980	160	2148.67	57.26	135.43	14.93	17.60
Mitchell	145	2111.33	43.37	127.10	18.46	24.40
Amsoy 71	120	1956.67	48.90	109.67	20.67	15.37
A-6242	165	1682.67	46.57	125.20	12.33	30.90
- - - - -						
EGF (5%)	---	235.46	9.67	9.67	0.85	10.10
CV (%)	---	5.88	14.72	5.92	3.45	15.63

Amsoy 71, although it was high yielding in double-cropping. Generally, early varieties were low yielding in main crop planting. Although A-6242 and A-5980 were late maturing varieties, high yield was not obtained from late varieties in main crop plantings. These varieties have long plant height and are usually susceptible to lodging. Though L-4106 has long plant height, it was found resistant to lodging.

Lodging resistance is an important agronomic characteristic for the determination of main crop varieties.

In conclusion, A-4595, A-3427, A-3127, Brich and S-3993 varieties can be grown as a main crop in the Cukurova region of Turkey. These varieties were high yielding. As can be see in Table 2, varieties belonging to Maturity Groups III, IV and V were very suitable for main crop planting in the Cukurova region.

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1) Progress in the regeneration of shoots from morphogenetic somatic hybrid tissue of soybean (+) *Glycine canescens*.

Previously, the Nottingham Group has reported the isolation and culture of protoplasts from *G. max* and perennial *Glycine* species, with plant regeneration from protoplast-derived tissues of *G. canescens* and *G. clandestina* (Davey and Hammatt, 1987). These procedures have now been used to obtain plantlet recovery from protoplasts of *G. argyrea* (Hammatt and Davey, 1989). Such plant regeneration studies form the basis for somatic hybridisation to circumvent sexual incompatibility between soybean and its wild relatives. In this respect, protoplast fusion experiments have continued in attempts to facilitate gene flow from perennial *Glycine* species into the genepool of *G. max*. Bifluorescent heterokaryons between *G. max* and *G. canescens* G1171 have been isolated using a Coulter Epics V flow cytometer (Hammatt et al., 1988) and cultured to produce somatic hybrid callus of *G. max* (+) *G. canescens* G1171. Hybridity has been confirmed by aspartate amino transferase isoenzyme analysis.

Somatic hybrid protoplast-derived tissues have been maintained on SC2 medium (Hammatt et al., 1986) for 15 months, during which time they have developed into glossy, green, nodular callus. Morphologically, this callus is similar to that which regenerates to form shoots in *G. canescens* G1171. However, to date, only bud-like structures have been recovered. These buds, 1-2 mm in diameter, possessed green protuberances reminiscent of leaf primordia, but failed to develop further upon subculture of the supporting callus to fresh SC2 medium.

Given the dwarf nature of *G. max* cv. HP-20-20, used as one of the parents in these fusion experiments, it was hypothesized that this characteristic might suppress the regeneration potential of the somatic hybrid tissues. Exogenously applied gibberellic acid (GA), known to promote shoot development, particularly of dwarfed plants, may stimulate shoot formation from somatic hybrid tissues. Assessments have been carried out to determine the levels of exogenously applied GA required to elicit a response in *Glycine*. Seeds of *G. canescens* G1699 were sown on hormone-free B5 medium (Gamborg et al., 1968) containing GA at 8 concentrations, ranging from 0.0 mg l⁻¹ to 100 mg l⁻¹. Such experiments revealed that 10.0 mg l⁻¹ was the lowest concentration of GA required to maximise seedling hypocotyl extension.

Buds and occasionally small shoots with 3-4 internodes, each about 1 mm in length, were obtained when GA at this concentration of 10.0mg/l- was added to SC2 medium. Buds resembled those obtained from protoplast-derived callus of G. canescens on GA-free SC2 medium, consisting of a whorl of scale-like structures with trichomes. Unifoliate leaves, each with distinct petioles and laminae, often developed from such scale-like structures. Buds and small shoots have been removed from the somatic hybrid callus and transferred to semi-solid SC6 medium, already known to support the development of buds into shoots in perennial Glycine spp. (Hammatt et al., 1987). Culture on this medium did not induce shoot extension, but while both buds and shoots remained alive, most of the bud scales and leaves became necrotic. In addition, swollen buds developed in the leaf axils of the shoots.

Transfer of these cultures to liquid SC6 medium in Woods flasks shaken at 80 rpm in the light (0.48 Wm⁻²) prevented leaf necrosis, but did not result in further growth of the buds. However, when 10 mg/l- GA was added to the liquid SC6 medium, leaf development ensued, producing unifoliate leaves 5 mm in length. Normal shoot extension to form an internode 10 mm in length has only been achieved to date from one bud. Additional manipulations of the culture medium components, particularly exposure to GA, may enable plants to be recovered from more of the somatic hybrid tissues. It is interesting to note that the regeneration potential of the perennial Glycine parent did not facilitate shoot recovery from somatic hybrid tissues, in contrast to the situation in many somatic hybrids, particularly members of the Solanaceae, where the regeneration potential of one partner is sufficient to induce shoot production from somatic hybrid tissues.

Given the long period of time in culture, somatic hybrid tissues of Glycine may have undergone changes at the nuclear level. Indeed, flow cytometric nuclear DNA analysis has confirmed that the somatic hybrid tissues between G. max and G. canescens contain only 90% of the expected parental DNA content (Hammatt and Davey, 1988). This indicates that part of the genome, possibly whole chromosomes, has been lost during the somatic hybridisation procedure or subsequent culture. Efforts will continue to improve the culture procedure for existing callus. In this way it is hoped to develop a medium to facilitate the rapid recovery of shoots from somatic hybrid tissues resulting from future protoplast fusion experiments.

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2) Towards the generation of interspecific somatic hybrids in the subgenus Glycine

In addition to intersubgeneric somatic hybridisation, intrasubgeneric hybrid production is being investigated within the subgenus Glycine. This approach should lead to reliable methods for culturing and regenerating somatic hybrid tissues, together with information on genomic behavior and interrelationships between wild species. Initial studies have included Glycine argyrea and G. clandestina, since it has been possible to regenerate plants from protoplast-derived tissues of both of these species using the same culture procedure (Hammatt et al., 1987, 1989).

Cotyledon protoplasts of G. clandestina, which have a strong chlorophyll autofluorescence, have been electrofused with fluorescein-labelled protoplasts isolated from hypocotyls of dark-grown seedlings of G. argyrea. The resultant fusion mixture was processed through the flow cytometer to isolate bifluorescent heterokaryons.

The culture procedure employed previously to stimulate callus formation and subsequent shoot regeneration from unfused, parental protoplasts was used for the sorted heterokaryons. However, heterokaryon-derived calli were friable, consisting of highly elongated cells. Such tissues bore little similarity to the compact, green, nodular callus normally produced by unfused protoplasts of G. argyrea and G. clandestina under similar culture conditions. This difference may result from heterosis and/or the increased ploidy levels of the hybrid cells.

Adjustments to exogenous hormone levels in the culture medium may overcome this problem, and facilitate the recovery of somatic hybrid plants.

To our knowledge, there have been no reports of somatic hybrid production in this subgenus. The genomes of any hybrid plants obtained from the current studies will be investigated both cytologically and at the molecular level. Such molecular studies will also be extended to organelle genomes, since an understanding of these genomes could be valuable in the introgression of genetic information from wild Glycine species into the cultigen.

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1) Screening of soybean germplasm for heat tolerance //

The effects of temperature on soybean yields often is underestimated despite reports of a significant relationship between soybean yields and growing season temperatures. Martineau et al. (1979) investigated temperature tolerance in soybeans and observed that maximum temperature during July and August was generally too high for optimum yields. They also noticed that genotypic differences in membrane thermostability were greatest in newly developed leaves. Thompson (1970) studied the impact of weather and technology in the production of soybean and noticed that highest yields have been associated with cooler than normal temperatures in July and August. Although the effects of temperature are difficult to separate from the effects of rainfall because of the tendency for intercorrelation, it appears that the identification of genotypes with heat tolerance is warranted.

This study was undertaken to carry out the RR5 regional project objective to evaluate the genetic variability among several soybean germplasms subjected to heat at various growth stages.

Materials and methods: The technique used for measuring heat tolerance of leaf tissue was as described for sorghum (Sorghum bicolor [L.] Moench) (Sullivan, 1972) with minor modifications for soybean (Martineau et al., 1979). Twenty soybean germplasms were planted randomly in a single-row plot, 6 m long and 90 cm wide in the field during the 1988 growing season. The same genotypes/germplasms were also planted in the greenhouse in the spring of 1988 and replicated 3 times. Leaf samples were taken at 2-week intervals during the season from the vegetative growth stage (V5) to reproductive growth stage (R4). Twelve leaflets were obtained by removing terminal leaflets from fully expanded trifoliolates at an uppermost node of 12 plants randomly selected in field plots, as well as from the greenhouse. In each germplasm, 12 leaflet samples were randomly divided into 4 groups, each containing 3 leaflets. From each 4-leaflet group, 14 leaf discs were cut with a 2-cm diameter cork borer and placed into a digestion tube. Two of the test tubes or samples were used for duplicate temperature treatments and the third one served as the control.

Prior to assay, leaf discs were washed several times with deionized distilled water distributed through a series of test tubes. This procedure was necessary to remove endogenous electrolytes adhering to tissue surfaces and to remove endogenous electrolytes released from cut cells at the periphery of the discs. After the final wash, the tubes were

drained for excess water and then covered with parafilm "M". The treatment tubes were placed in a temperature-regulated water bath at 50 ± 1 C for 15 min while the control was maintained at 25 C. Tubes were then removed from the water bath and 30 ml of deionized distilled water was added to each and incubated overnight for 18 h at 10 C to allow the diffusion of electrolytes from the discs. Then, initial conductance readings were made at 25 C with use of a Yellow Springs Instrument (YSI) Model 35 Conductance meter and Model 3403 conductivity cell. Upon completion of this measurement, the control and treatment tubes were covered with cotton wool and autoclaved at 110 C, with 1.4 kg pressure for 10 min to completely kill the leaf tissue. Final conductance measurements were taken after all tubes were cooled to 25 C.

Determination: The degree of injury induced as a result of the temperature treatment was calculated as follows:

$$\% \text{ injury} = \left[1 - \frac{1 - (T_1/T_2)}{1 - (C_1/C_2)} \right] \times 100$$

where T and C refer to treatment and control, respectively, and the subscripts 1 and 2 of T and C refer to initial and final conductance, respectively. The ratio of initial conductance (i.e., T_1/T_2) is a relative measure of the amount of electrolyte leakage induced by the elevated temperature and is assumed to be proportional to the amount of "injury" induced in cellular membranes. The inclusion of controls in this assay provides a measure of spurious electrolyte leakage due solely to the cutting and subsequent handling of the leaf discs and the 18-h storage at low temperature. Consequently, the calculated injury values would reflect only that induced by the elevated temperature treatment.

Results and discussion: Percentage heat injury varies significantly at different growth stages, with the highest heat injury occurring at flowering to pod, except for 'Guelph', 'Emperor' and 'Sanga', in which heat injury increased as plant growth progressed (Table 1). This result agrees with the work of Bouslama and Schapaugh (1984) who studied stress tolerance in soybeans and observed that the maximum significant cultivar separation occurred at the flowering stage of R1.

Inconsistent results between greenhouse and field experiments were observed in this study (Fig. 2), probably due to high disease and insect infestation in the greenhouse. Besides, Wehner and Watschke (1981) also noticed that testing with greenhouse and growth-chamber-grown plants showed obvious differences in heat tolerance. Despite these differences in results, however, data averaged over all growth stages showed that Williams was significantly more heat tolerant than all other germplasm (Figs. 1 and 2), whereas 'Emerald', 'Jefferson', 'Emperor', and 'Fuji' were less heat tolerant in comparison with other germplasm (Fig. 1).

<u>Tolerant</u>	<u>Moderately tolerant</u>	<u>Sensitive</u>
Williams 79	Funk Delicious	Imperial
Kura	Oakland	Guelph
Pella	Wolverine	Fuji
Sato	Columbia	Emperor
	Kanrich	Jefferson
	Sanga	Emerald
	Kim	
	Willomi	
	Jogun	
	Ware	

The results of this experiment will provide germplasm and parental lines to plant breeders to develop new soybean genotypes tolerant to heat and drought.

Research work is also in progress on 20 additional soybean germplasms that have been planted in the greenhouse. Testing started last November, to be completed by the end of December, 1988. Studies on this project will continue in spring 1989. The first part of this study will be presented at Southern Branch of American Society of Agronomy, Nashville, TN Feb. 5-7, 1989.

Table 1. Mean heat-induced injury values of 20 soybean germplasms at different growth stages.

Germplasm	Growth stages		
	V5	R1-R2	R4
	-----% injury-----		
Jogun	54.45 b	68.90 a	34.90 c
Kanrich	36.80 c	63.30 a	48.00 b
Wolverine	30.20 c	66.20 a	39.20 b
Willomi	21.25 c	87.50 a	47.70 b
Oakland	19.85 b	56.60 a	57.05 a
Pella	18.98 c	57.65 a	33.30 b
Williams 79	26.60 b	39.95 a	25.40 b
Kim	47.65 b	60.65 a	46.90 b
Kura	25.20 b	45.00 a	39.15 a
Columbia	44.60 a	50.00 a	46.70 a
Fuji	53.55 b	69.30 a	60.10 b
Guelph	49.60 c	60.00 b	70.40 a
Emerald	53.60 b	72.80 a	70.55 a
Imperial	48.80 b	84.75 a	42.45 b
Ware	56.00 b	69.30 a	41.35 c
Emperor	40.80 c	57.85 b	89.85 a
Funk Delicious	36.35 b	47.00 a	38.60 b
Jefferson	53.60 b	80.10 a	57.70 b
Sanga	32.70 c	52.90 b	68.65 a
Sato	32.50 b	55.55 a	23.75 c

Means within a row followed by the same letter(s) are not significantly different at the 5% level of probability according to DMRT.

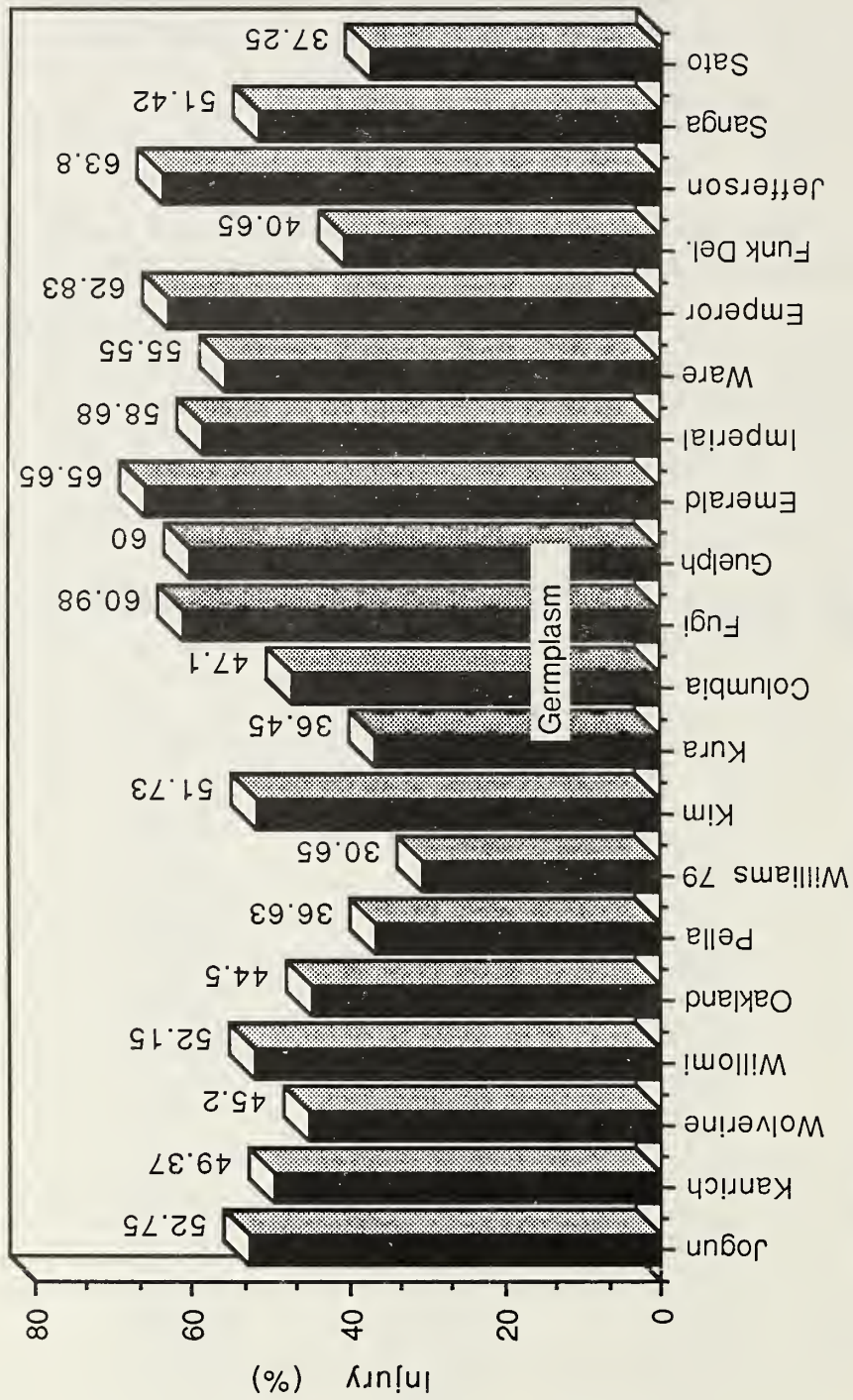


Fig 1. Mean heat injury of 20 soybean germplasm (1988).

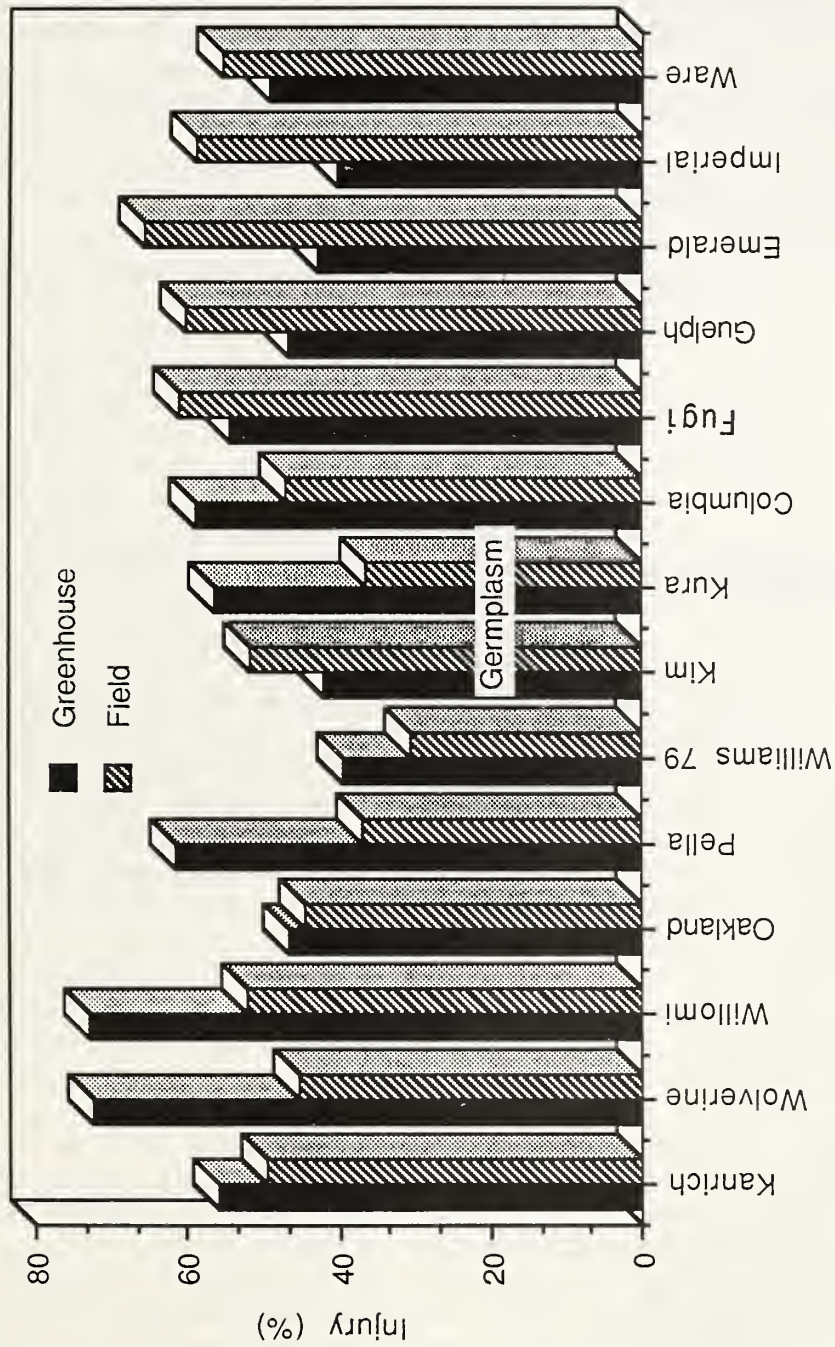


Fig 2. Percent injury of 14 soybean germplasm in field and greenhouse (1988).

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2) Sulfur-containing amino acids and protein content in selected soybean progenies.

Summary: Three soybean crosses (X-1026, X-1065, X-1066) were studied in F2 and F3 generations for variability in levels of protein and sulfur-containing amino acids (methionine and cysteine). Cross X-1065 ('Tracy' x 'Essex') showed an increase in protein content of approximately 2% while protein decreased in X-1065 ('Dare' x 'Forrest') and X-1066 ('Ogden' x Tracy). Methionine and cysteine levels in parents and F3 progenies of X-1065 showed significant differences among progenies while only two or three progenies were different in X-1065 and X-1066. All crosses showed a significant negative correlation between the level of protein and sulfur-containing amino acids. Among the three crosses, X-1026 showed a greater variation in sulfur-containing amino acid and protein content between the F2 and F3 generation. Parent-offspring regression analysis suggests that the sulfur-containing amino acids are a heritable trait.

Introduction: Soybean is one of the most important sources of protein in the nutrition of populations that derive most of their protein needs from plant foods. However, the nutritional value of soybean protein is limited by a deficiency in sulfur-containing amino acids, both methionine and cysteine (Evans and Boulter, 1980; Spackman and Stein, 1958; Yu and Bliss, 1978; Kelly, 1971). The reported average levels (mg/100 g food) of methionine and cysteine in soybean are 525 and 552, respectively, and such low levels of sulfur-containing amino acids give the soybean protein a very low chemical score of 47 (Krober, 1966). In recent times, increasing interest has been shown in the possibility of breeding soybeans with a higher protein content, but the question has been

raised as to whether already low levels of sulfur-containing amino acids would decline, if the protein content is increased (Adam, 1973; Yu and Bliss, 1978). On the other hand, one may be able to increase the levels of these amino acids simultaneously while increasing the protein quantity by proper selection of the cultivars for breeding.

This paper describes the study on genetic variability for protein and sulfur-containing amino acids in F2 and F3 generations and estimates the heritability by parent-offspring regression.

Materials and methods: Amino acid analysis was performed on three crosses (X-1065, Dare x Forrest; X-1065, Tracy x Essex; X-1066, Ogden x Tracy) that were spaced-planted in May 1981 and 1982 at Alabama Agricultural and Mechanical University's research farm as the F2 and F3 generations, respectively. In both generations, limited numbers of progenies were studied in each cross.

Protein content was estimated by determining the nitrogen content of mature dry seeds, by the Micro-Kjeldahl method (AOAC, 1975) and multiplying the nitrogen value by 6.25. Sulfur-containing amino acids were analyzed by performing acid oxidation Method (Moore, 1963; Moore and Stein, 1966) with slight modifications. One hundred microliters of samples were injected into a Beckman 118B amino acid analyzer. The levels of methionine and cysteine acid were expressed as either mg/100 g samples or g/100 g protein. The calculations were based on the peak areas of external standards injected between every 3 or 4 samples (Moore and Stein, 1966). Statistical analysis was performed and correlation coefficients were determined between F2 and F3 progenies.

Results: The methionine and cysteine acid levels for parents and F3 progenies in all three crosses are presented in Table 1. Significant differences among progenies from both parents were observed in all three crosses. This significant increase in F3 mean over parent was due to the increase of methionine coming from both parents but for cysteine the significant increase probably came from parent Forrest. Few progenies in the cross Tracy x Essex had significantly higher methionine and cysteine than either of their parents (Table 1). In the Ogden x Tracy cross, a significant difference was found among progenies 1 and 2, compared with either of its parents for methionine. The cysteine in protein and sample showed no significant difference between their parents and the significant differences found in all three crosses suggested that some sort of gene action effect (additive, transgressive, or both) was taking place (Porter, 1972). For the Ogden x Tracy cross, approximately 50% of the progenies showed an increase in the sulfur-containing amino acids within the protein as well as within the samples, showing that the additive effect was occurring. In all crosses, for each variable observed, the coefficient of determination (R^2) was high. The amount of variation that could be accounted for was approximately 70 to 90%, except for cysteine in sample in cross Ogden x Tracy, in which approximately 50% of the variation could be accounted for. The data suggested that the inheritance of methionine-cysteine is genetically controlled, which is in agreement with Leleji (1972).

Both sulfur-containing amino acids (methionine and cysteine) within

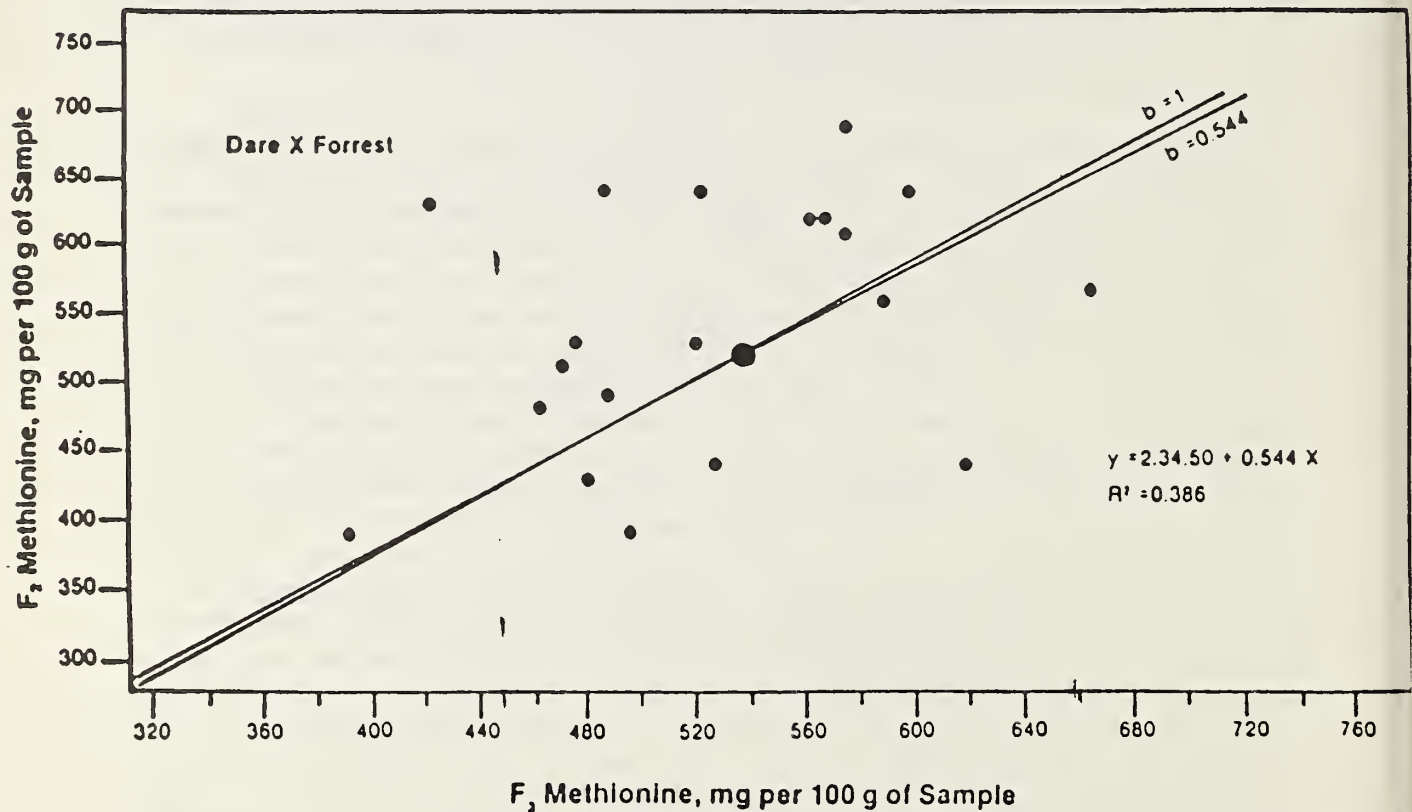
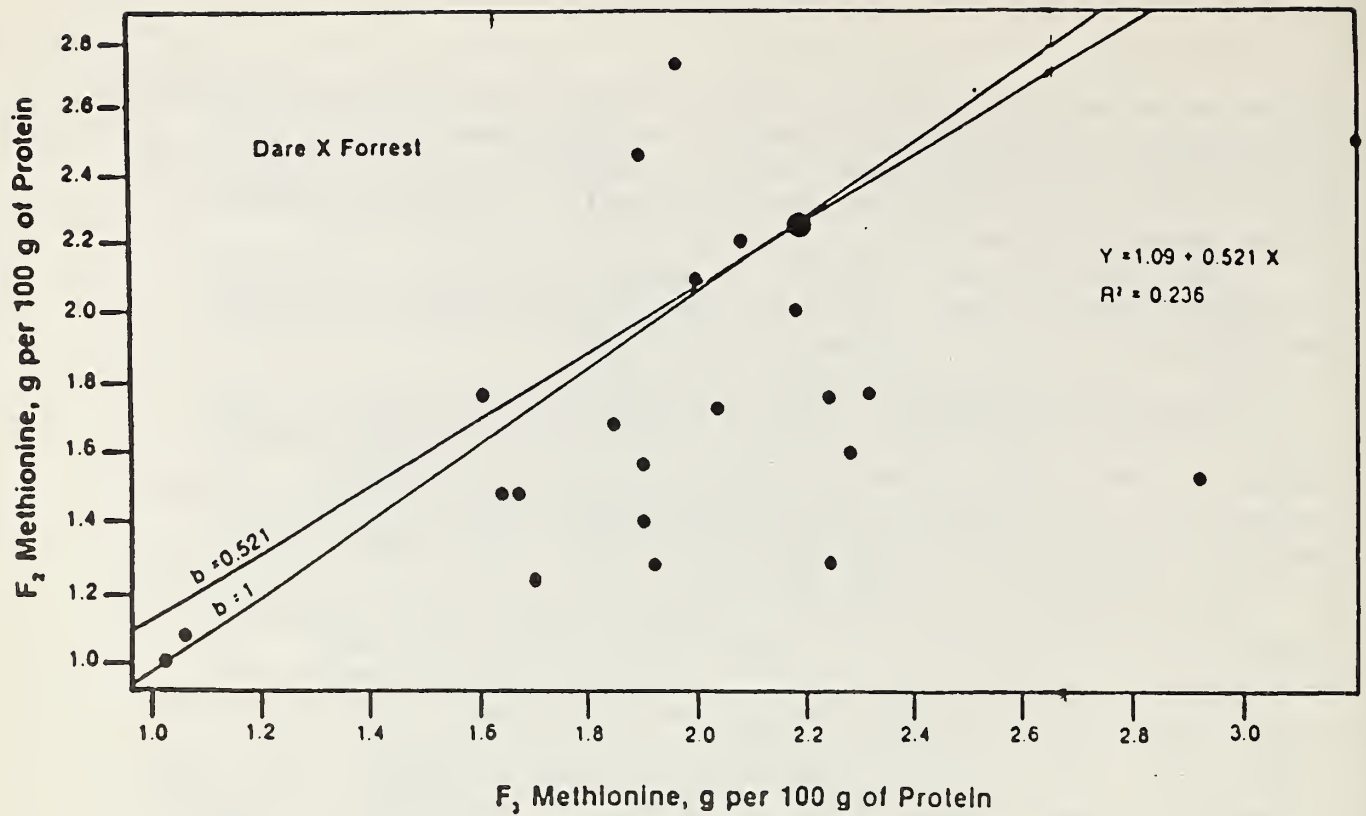


Fig. 1. Parent-offspring regression analysis between F_2 milligrams of methionine per 100 grams of sample and F_3 milligrams of methionine per 100 grams of sample for Dare x Forrest cross.

the protein as well as within the sample were found to be significantly correlated with protein at the 0.01 and 0.05 probability level (Table 2). The relationships among sulfur-containing amino acids and protein content between F2 and F3 generations of all three crosses are presented in Table 3. Among these crosses and in cross 1026 (Dare x Forrest) methionine within protein and sample showed a significant correlation with protein and cysteine. Cross 1066 showed significant correlation at the 0.05 level for protein and methionine in the sample. There was no significant correlation found between any forms of sulfur-containing amino acids and protein in X-1065.

For all crosses studied, protein was negatively related with all sulfur-containing amino acids except for cysteine in sample in cross Dare x Forrest, which had a very low positive correlation of 0.058 (Table 2). The highly significant negative correlations between protein and the two sulfur-containing amino acids for Dare x Forrest were observed in this study. Highly significant and negative correlation between protein and the sulfur-containing amino acids was observed in the Tracy x Essex cross. This negative correlation is in agreement with the previous report by Adams (1973).

The parent-offspring regression analysis was performed on cross Dare x Forrest due to the significant correlation obtained. This type of analysis is an alternative approach to heritability used between F2 and F3 generations. The parent-offspring regression analysis indicated high regression coefficient for methionine (protein) and methionine (sample) of 0.541 and 0.544, respectively, between F2 and F3 generations, which suggested that methionine could be a heritable trait (Fig. 1). The R² values for the interaction shown between the two generations for methionine (protein) and methionine (sample) were 0.236 and 0.386, respectively, indicating that approximately 25 to 40% of the variation can be explained based on genotypic influence of the progenies. However, the heritability for cysteine (sample) was low, suggesting that the scope of genetic advancement in improving cysteine is limited for this cross.

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Table 1. Mean and range of methionine and cysteine (per 100 g of protein and sample) of parent and F3 segregates in three soybean crosses.

Population Cross	Per 100 g protein		Per 100 g sample	
	Methionine	Cysteine	Methionine	Cysteine
Dare X1026	1.02	1.31	356.8	457.2
Forrest	1.07	2.10	392.9	770.8
F3 mean	1.96	2.03	544.2	528.3
F3 range	1.00-3.21	1.61-2.73	422.3-798.0	464.0-680.0
N	20	20	20	20
R2	0.915	0.799	0.863	0.752
Tracy X1065	1.63	2.32	471.9	669.7
Essex	1.17	1.50	390.0	497.8
F3 mean	3.24	2.73	884.2	735.6
F3 range	2.03-8.55	0.73-8.27	627.8-1652.5	231.6-1559.4
N	18	18	18	18
R2	0.719	0.706	0.915	0.915
Ogden X1066	1.04	1.83	316.8	556.8
Tracy	1.63	2.32	444.9	669.7
F3 mean	2.09	2.26	540.9	601.6
F3 range	1.12-3.44	1.88-2.58	357.7-975.2	483.4-708.4
N	10	10	10	10
R2	0.739	0.704	0.750	0.473

Table 2. Simple correlation coefficients among sulfur-containing amino acid and protein content in the F3 generation for three soybean crosses.

Variable	Cross	2	3	4	5
1. Protein	X-1026	0.058	-0.306*	-0.753**	-0.763**
	X-1065	-0.141	-0.193	-0.568**	-0.647**
	X-1066	-0.018	-0.144	-0.784**	-0.450**
2. Cys S	X-1026		0.341*	0.532**	0.191
	X-1065		0.863**	0.830**	0.730**
	X-1066		0.477*	0.606**	0.388*
3 . Met S	X-1026			0.448**	0.777**
	X-1065			0.739**	0.806**
	X-1066			0.401*	0.897**
4. Cys P	X-1026				0.721**
	X-1065				0.943**
	X-1066				0.611**
5. Met P					

**, * Significant at 0.01 and 0.05 level of probability, respectively.

S = mg of amino acid/100 g sample; P = g of amino acid/100 g protein.

Table 3. Correlation coefficients among sulfur-containing amino acid and protein content between F2 and F3 generations of three crosses.

		F3					
Variables		Cross	Protein	Cys S	Met S	Cys P	Met P
F2	Protein	X-1026	0.226	0.077	0.049	0.052	0.033
		X-1065	0.049	0.278	0.35'	0.314	0.338
		X-1066	0.215	0.066	0.644*	0.016	0.624
	Cys S	X-1026	0.083	0.533*	0.065	0.325	0.038
		X-1065	0.163	0.162	0.185	0.199	0.189
		X-1066	0.435	0.460	0.109	0.427	0.115
	Met S	X-1026	0.419*	0.136	0.622**	0.471*	0.624**
		X-1065	0.031	0.223	0.169	0.220	0.167
		X-1066	0.186	0.323	0.154	0.023	0.164
	Cys O	X-1026	0.000	0.498*	0.148	0.327	0.080
		X-1065	0.081	0.058	0.099	0.071	0.081
		X-1066	0.462	0.297	0.334	0.348	0.325
	Met P	X-1026	0.401*	0.182	0.503*	0.437*	0.486*
		X-1065	0.042	0.128	0.091	0.111	0.076
		X-1066	0.032	0.216	0.438	0.005	0.433

**, * Significant at 0.01 and 0.05 level of probability, respectively.

S = mg of amino acid/100 g of sample; P = g of amino acid/100 g of protein.

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1) Sterile and semi-sterile mutants in the Genetic Type Collection. //

In the USDA Genetic Type Collection, there are a number of entries that are described primarily as sterile. Of these, 14 are listed as male sterile, although some of them are known to have a fairly high degree of female sterility. Each of these is controlled by a single recessive mutant gene, ms1 to ms6, including six mutations to ms1, three to ms3, and two to ms4, and one is listed as partially male sterile, controlled by gene msh (Palmer and Kilen, 1987; Bernard, 1988). There are five other T-strains described as sterile (actually near-sterile, since in some cases a few pods are produced). For each, the genetic and cytogenetic causes of the sterility have been described, asynapsis for T241 st2 and T242 st3 (Hadley and Starnes, 1964), desynapsis for T258 st4 and T272 st5 (Palmer, 1974; Palmer and Kaul, 1983), and structural sterility for T269 fs1 fs2 (Johns and Palmer, 1982).

Other mutations to sterility are often observed in breeding populations, and a collection of these has been assembled at Urbana and is being placed in the Genetic Type Collection so as to be readily available for study. A number of these have been observed in segregating plant progenies, and in many cases inheritance is as a single gene recessive. Test crosses to lines with known genes have not been made so it is not known if they are controlled by the same or different genes. The following table summarizes results from observing progenies of fertile plants for the mutants segregating as single recessive genes. The few seeds available from the near-sterile and semi-sterile plants produce erratic results, often including many fertile plants and plants with traits suggesting outcrossing.

The rest of the semi-steriles in this collection do not segregate clearly in 3:1 ratios. Some of them breed true for a moderate partial sterility (or don't set pods well for whatever reason), some appear to be segregating 1:1 (and might be caused by chromosome inversions), and others have been difficult to classify on a single plant basis. These will be observed at Urbana in 1989 and some of these may also be added to the Type Collection.

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2) Screening the *Glycine clandestina* Wendl. collection for shoot regeneration from leaf explants✓

Glycine clandestina Wendl. is a wild perennial relative of the cultivated soybean, *G. max* (L.) Merr. Plant regeneration from various explant sources have been reported with *G. clandestina* (Hammatt et al., 1986, 1987 a, b; Hymowitz et al., 1986; Jones et al., 1988). However, in each study a limited number of accessions were examined. Furthermore, a maximum regeneration efficiency of 83% from proximal halves of cotyledons (Hammatt et al., 1986) and 79% from leaf explants (Hymowitz et al., 1986) were reported. In this study we examined a total of 33 accessions of *G. clandestina* for their ability to regenerate plants from leaf explants.

Materials and methods: The 33 accessions of *G. clandestina* utilized were maintained at the University of Illinois. Seeds were germinated, leaf explants were prepared and cultured according to Hymowitz et al. (1986).

Results and discussion: The results are summarized in Table 1. Only six accessions regenerated shoots. PI 505163 (also identified as IL727 or G1826) displayed the highest rate of regeneration (100%) and had the maximum number of shoots (15) per explant. The shoots were easily rooted and transferred to the greenhouse as described by Hymowitz et al. (1986). The results were reconfirmed by reculturing the leaves of those accessions that showed the ability to regenerate. We hope that the use of a high regenerating genotype of *G. clandestina* will help simplify plant generation problems often encountered in transformation and various screening systems.

Table 1. Screening of Glycine clandestina accessions for regeneration from leaf explants; 15 leaf explants cultured per accession.

PI	No. of explants produced callus	No. of explants formed shoots	No. of shoots/explant
233138	15	0	0
246590	15	0	0
248252	15	0	0
255745	15	0	0
339656	15	0	0
339660	15	0	0
378870	10	0	0
400967	15	0	0
429809	15	0	0
440944	15	1 (7%)	1
440945	15	0	0
440947	0	0	0
440948	9	4 (27%)	3
440949	15	0	0
440950	15	0	0
440951	5	0	0
440952	15	1 (7%)	3
440954	14	0	0
440955	15	0	0
440957	15	0	0
440958	15	12 (80%)	4
440959	15	0	0
440960	15	0	0
440961	15	0	0
440966	5	0	0
440968	1	0	0
440969	8	0	0
440970	15	1 (7%)	1
446943	15	0	0
483194	10	0	0
505161	15	0	0
505162	11	0	0
505163	15	15 (100%)	15

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3) Isolation of three different triple null genotypes for seed protein components of soybeans//

The research reported herein is part of a long range study to develop soybean seed lacking or having very low amounts of the following proteins: Kunitz trypsin inhibitor, urease, lectin, lipoxxygenase-1, and B-amylase. An attempt was made to develop triple null genotypes for both the Kunitz trypsin inhibitor (ti) and urease (sun) in reciprocal combinations with the lack of lectin (le), lipoxxygenase-1 (lx1) or B-amylase (spl).

Materials and methods: Three sets of reciprocal crosses were made between the double null genotype lacking the Kunitz trypsin inhibitor and seed urease (titi sunsun), and the other three double nulls obtained by Prischmann and Hymowitz (1988): titi lele, ti ti lx1lx1, and titi splspl. Analysis of F2 seed was carried out as described by Prischmann and Hymowitz (1988). Since the partial seed technique was used to characterize the genotype of seeds, it was necessary, in some cases, to use embryo-rescue techniques to produce the next generation (Singh et al., 1987). For the titi sunsun splspl genotype none of the F2 triple nulls could be recovered as a plant. However, triple nulls of this genotype were recovered from F3 seed of a titi Sun--- splspl plant.

Table 1. Recovery of triple null genotypes for reciprocal crosses between various double null genotypes.

Plants	Total of F2 seed	Number of triple null seed
2	<u>titi</u> <u>sunsun</u> <u>SplSpl</u> x <u>titi</u> <u>SunSun</u> <u>splspl</u> 54	4 [†]
2	<u>titi</u> <u>SunSun</u> <u>splspl</u> x <u>titi</u> <u>sunsun</u> <u>SplSpl</u> 36	3 [†]
2	<u>titi</u> <u>sunsun</u> <u>LxlLxl</u> x <u>titi</u> <u>SunSun</u> <u>lxllxl</u> 48	1
5	<u>titi</u> <u>SunSun</u> <u>lxllxl</u> x <u>titi</u> <u>sunsun</u> <u>LxlLxl</u> 186	19 (5 [†])
1	<u>titi</u> <u>sunsun</u> <u>LeLe</u> x <u>titi</u> <u>SunSun</u> <u>lele</u> 54	6

[†]Plants were not recovered from seed.

Results: Triple recessive genotypes were obtained for five out of the six null combinations (Table 1). One F1 plant from the cross titi SunSun lele x titi sunsun LeLe has been obtained. Thus, the missing null genotype titi sunsun lele most probably will be obtained in F2 generation. Evidence of linkage has not been found in any case. None of the triple nulls seem to cause seedling inviability or to be lethal to the plant.

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 4) Development of culture media for in vitro propagation of soybean x wild perennial Glycine species hybrids.

Efforts to exploit the wild perennial relatives of the soybean (subgenus Glycine) as a source of useful new genes to expand the soybean germplasm base have heretofore proven unsuccessful. While at least six different intersubgeneric crosses have been reported (Broué et al., 1982; Newell and Hymowitz, 1982; Brown et al., 1985; Singh and Hymowitz, 1985; Newell et al., 1986; Singh et al., 1987), rates of crossability are extremely low due to poor gene pairing (Palmer and Hadley, 1968; Ladizinsky et al., 1979). Post-fertilization problems are compounded by the stress of environmental variables such as disease, chemicals, light, etc., in the greenhouse setting. In vitro fertilization and ovary culture could provide a measure of control over the development of the resulting seed and environmental variables. An investigation of the feasibility of utilizing in vitro fertilization and ovary culture to facilitate wide crossing with Glycine species led to the formulation of a nutrient culture medium capable of sustaining embryo development through early heart stage.

Experimentation began with the assessment of three documented media formulations. Media were evaluated as their ability to effect fertilization of G. max by G. tomentella and to promote normal seed/pod development. The formulations include:

- * B-5 medium of Gamborg et al. (1968) as modified by Newell and Hymowitz (1982) for embryo rescue of G. max x G. tomentella hybrids.
- * Medium for ovary growth of self-fertilized wild perennial Glycine species by Silvoy (1984).
- * Tilton's in vitro pollination medium (1983) developed to effect G. max x G. max fertilization.

Flower buds to be cultured on each medium were harvested from healthy soybean [strain T31] stocks 1 to 2 days before anthesis. Buds were chosen from the middle sector of plants in the second to third week of the flowering cycle. Selections were sterilized in a 25% solution of Clorox with 4 drops of Tween 80 for 9 min. During emasculation, flowers were left intact as much as possible. Only anthers and corolla were removed through a small window cut in the calyx, adjacent to the stigma. In addition, every effort was made to include most of the pedicel. Explants were cross-pollinated just prior to culturing and incubated at 26 C with 16 hr of cool white fluorescent light provided daily.

Glycine max [strain T31] pistils, hand-pollinated by G. max [cv. Williams], and unpollinated T31 pistils were cultured as checks.

As a result of this initial evaluation, Silvoy's medium (1984) elicited the most positive response. Wide fertilization was achieved and the most normal seed/pod development ensued over the following 4 weeks.

While embryos had deteriorated in hybrid seed, thriving embryos were recovered from G. max x G. max checks.

In the second phase of experimentation, Silvoy's medium (1984) was varied for carbon/nitrogen sources and amounts. The addition of 1 g/l L-malic acid, 20 mg/l ascorbic acid, and a modified version of the KT vitamin mixture (Horn et al., 1983) significantly improved pod growth and health and promoted normal embryonic development through the globular stage in wide crosses.

This modification was then further varied for hormone types, levels and ratios. Hybrid embryos at early heart stage were recovered from a modification containing 0.05 mg/l BAP and 2.5 mg/l gibberellin plus 5 g/l activated charcoal.

This preliminary study demonstrates the potential for use of in vitro fertilization and immature seed culture to exploit valuable characteristics within the wild perennial Glycine species. Further refinement of media is needed. Perhaps methods to directly culture either seed or embryos should be considered.

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5) Management of the USDA wild perennial Glycine collection/

Under a specific cooperative agreement between the University of Illinois and the USDA, the wild perennial Glycine collection currently is being maintained at the University of Illinois. The objectives of the project are as follows: 1) to enlarge the collection of the wild perennial species of Glycine so as to make it include all such species and encompass a wide selection of geographic variants; and 2) to preserve seeds of each accession and make them available for research purposes. The species in the collection, somatic chromosome number, number of accessions and origin is presented in Table 1. The various activities of the cooperative agreement are presented below.

Multiplication: The accessions are very difficult to multiply. The plants primarily are adapted to the tropics and subtropics. They grow very slowly, have a procumbent habit of growth, most are photoperiodic, and produce few pods that upon maturity shatter their seed. Seed multiplication is undertaken in the greenhouse complex next to Turner Hall and on the South Farm Experiment Station.

In the greenhouse the plants are maintained in 6-inch pots (soil, a 1:1:1 mixture of clay loam:peat:sand). From September to mid-May, the plants are grown in the greenhouse. From mid-May to mid-September, the plants are moved to just outside the greenhouse in a protected area.

In the greenhouse, the light source is provided by halogen lights. The number of hours of light the wild perennial Glycine plants receive is based upon the flowering requirements of soybean plants growing in the same greenhouse. Specific plants of wild perennials are induced to flower by placing them in a dark chamber for 14 days, during which time they receive only 9 hours of light per day. The plants are fertilized every two weeks with a soluble fertilizer. As needed, Diazinon, Avid, Malathion, Benlate, and yellow gummed strips are used to protect the plants from mealy bugs, white flies, spider mites, fungus gnats, and powdery mildew. Bamboo poles are placed in the pots to allow the plants to climb. All pots and stakes are sterilized before use. The greenhouse floor and benches are vacuumed about once a month as a sanitation measure. Each working day, mature pods are collected from the plants and individually shelled.

From mid-May to mid-September, the wild perennial plants are moved to a protected area just outside the greenhouse. During this period, the greenhouse is thoroughly cleaned and fumigated. Unfortunately, in mid-September the plants growing outside the greenhouse have to be cut back and returned to the greenhouse. It is exactly this period of time in which many of the wild perennial Glycine plants start to flower.

This year, plants were grown on the South Farm Experiment Station in the field under a movable greenhouse placed on tracks. The plastic-covered greenhouse is 127 feet long and 33 feet wide. In early March, the seeds were germinated in a sandbench in the Turner Hall greenhouse complex and about a month later the seedlings were transplanted to 3-inch x 3-inch pots. In late May, the young plants were moved into the field. About 440 plants representing 157 accessions were planted in checkerboard fashion 36 inches apart within and between rows. Soon after planting, Dacthal was surface applied. In addition, the field was rotohoed twice and hand hoed to reduce weeds. The plants were fertilized about every three weeks with a soluble fertilizer. A 4-foot bamboo stake was placed by each plant to allow it to climb. An irrigation pipe was placed lengthwise across the middle of the field. It contained 4-foot risers with 6 birdies spaced 20 feet apart. The plants were watered as needed. During inclement weather the greenhouse is rolled over the plants. The greenhouse protected the plants from a deep frost until mid-November.

Table 1. Wild perennial Glycine collection, 1988.

Species	2n	Number of accessions	Origin
<u>G. arenaria</u>	40	3	Australia
<u>G. argyrea</u>	40	3	Australia
<u>G. canescens</u>	40	41	Australia
<u>G. clandestina</u>	40	49	Australia
<u>G. curvata</u>	40	5	Australia
<u>G. cyrtoloba</u>	40	18	Australia
<u>G. falcata</u>	40	8	Australia
<u>G. latifolia</u>	40	16	Australia
<u>G. latrobeana</u>	40	11	Australia
<u>G. microphylla</u>	40	18	Australia
<u>G. tabacina</u>	40	17	Australia
	80	70	Australia, Taiwan, Japan, Mariana, Fiji, Tonga, Vanuatu, New Caledonia
	Unknown	31	
<u>G. tomentella</u>	38	3	Australia
	40	11	Australia, Papua New Guinea
	78	50	Australia, Papua New Guinea
	80	49	Australia, Papua New Guinea, Philippines, Taiwan
	Unknown	89	
TOTAL		492	

Preservation: Approximately 240 wild perennial Glycine accessions, each containing 50 seed per packet, were sent to the National Seed Storage Laboratory, Ft. Collins, CO, for long-term storage. Another emergency set, containing 10 seed per packet, was sent to R. L. Bernard, USDA/Urbana. Currently, the wild perennial Glycine accessions are maintained in envelopes in a milk cooler, set at 4 C, located in C117, Turner Hall.

Morphological data: All morphological data recorded during the past 10 years were sent to Gail Juvik, Assistant Curator, Soybean Germplasm Collection. She will place the data in the GRIN system.

Herbarium specimens: Voucher specimens of all accessions have been placed in the Crop Evolution Herbarium (CEL).

Distribution of seed: The distribution of seed is divided into parts, foreign and domestic. For foreign distribution, the packets of seed are sent to R. L. Bernard and processed by him. The distribution of seed for domestic use is handled within the Department of Agronomy.

During the year, 20 seed requests were received. A total of 182 packets of seed were shipped. The foreign requests came from Iraq, U.K., South Korea, and Taiwan.

Ordering seed: Requests for wild perennial Glycine seed should be directed to T. Hymowitz, Department of Agronomy, University of Illinois, 1102 South Goodwin Avenue, Urbana, IL, 61801, USA. If available, 5 to 10 seed per accessions are shipped.

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6) Exploration for wild perennial Glycine species on Taiwan.

From March 29 through April 16, 1988, I participated in a wild perennial Glycine collecting trip on Taiwan. The trip was sponsored by the Office of International Agriculture, University of Illinois, and USAID/USDA Grant No. SG-86-CRSR-2-2865. On Taiwan the collecting phase was coordinated by Dr. S. H. Cheng, and in part funded by the Council of Agriculture, Taipei. Members of the Glycine collecting team included Dr. M. S. Yeh, National Chung-Hsing University, Taichung; Drs. J. S. Hsieh and Y. C. Huang, National Taiwan University, Taipei; Mr. K. F. Chen, Kaohsiung District Agricultural Improvement Station, Pintung; and Dr. S. H. Cheng, Council of Agriculture.

Four accessions of G. tomentella were collected in southwestern Taiwan (Table 1). The tomentellas were found growing on stabilized sand dunes. Ten accessions of G. tabacina were collected from six islands of the Pescadores Archipelago (Table 1). The low lying windswept groups of islands lie in the Strait of Taiwan between Taiwan and the People's Republic of China. All tabacinas found were growing on rocky sites.

Curiously, no *tabacinas* were seen in southwestern Taiwan and no *tomentellas* were seen on the Pescadores Archipelago.

All accessions currently are growing in the greenhouse. Thus far, three accessions have flowered and are tetraploids ($2n=80$, chromosome counts by R. J. Singh).

Table 1. Accessions of *Glycine tomentella* and *G. tabacina* collected on Taiwan, 1988.

Species	IL [†]	2n	Origin
<i>G. tomentella</i>	871	80	Maopitou
<i>G. tomentella</i>	872	--	Ta Kuang
<i>G. tomentella</i>	873	80	Paisha
<i>G. tomentella</i>	874	--	Haiku
<i>G. tabacina</i>	875	80	Penghu Is., Pescadores
<i>G. tabacina</i>	876	--	Paisha Is., Pescadores
<i>G. tabacina</i>	877	--	Hsiaomen Is., Pescadores
<i>G. tabacina</i>	878	--	Hsiyu Is., Pescadores
<i>G. tabacina</i>	879	--	Hsiyu Is., Pescadores
<i>G. tabacina</i>	880	--	Penghu Is., Pescadores
<i>G. tabacina</i>	881	--	Chumei Is., Pescadores
<i>G. tabacina</i>	882	--	Chumei Is., Pescadores
<i>G. tabacina</i>	883	--	Chumei Is., Pescadores
<i>G. tabacina</i>	884	--	Ghebay Is., Pescadores

[†]IL numbers are temporary University of Illinois accession numbers.

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7) Induction of amphidiploids through colchicine treatment in the genus *Glycine*.

Chromosome doubling is a necessary step to overcome sterility problems in wide hybrids in the genus *Glycine*. Applications of colchicine to seeds or parts of whole plants is a common method for inducing polyploidy in various taxa. Chromosome doubling in wild perennial *Glycine* species previously has been reported. Palmer and Hadley (1968) noted that the chromosomes of *G. latifolia* (Benth.) Newell and Hymowitz (formerly *G. tomentosa* Benth.) could be doubled by applying a 0.5% colchicine-lanolin paste to the cotyledonary nodes prior to the opening of the cotyledons. Cheng and Hadley (1983) applied 0.1% aqueous colchicine solution to absorbent cotton wrapped at the meristematic regions of young seedlings or a scion of a graft with the apical shoot bud removed. Unfortunately, in certain wide *Glycine* hybrids the chromosomes are extremely difficult to double by conventional *in vivo* methods. Herein we report on the use of a tissue culture method that successfully was utilized to induce chromosome doubling in such hybrids.

Materials and methods: A sterile hybrid H310 [*G. latifolia* ($2n=40$, B1B1) x *G. canescens* ($2n=40$ AA)] was used throughout this study. Three treatments were employed.

1. Axillary buds: Absorbent cotton was wrapped at the axils of young cuttings with their terminal shoot bud removed. The cotton wrappings were soaked with 0.1% aqueous colchicine solution once a day for 5 consecutive days. The cuttings were grown in 6-inch pots in the greenhouse.

2. Roots: Young cuttings were grown in Magenta boxes containing sterile vermiculite and peat-moss (3:1). Approximately 10 ml of a 0.1% aqueous colchicine solution was applied to the roots once every 3 days for a total of 4 applications.

3. Shoot-tip culture: Growing shoot-tips (apical or axillary) of 1.5-2.0 cm long were surface-sterilized in 20% commercial bleach (1% sodium hypochloride) for 15 min., followed by three sterile water rinses under aseptic conditions. The shoot tips then were cultured on shoot-proliferation medium (medium 'C' of Singh et al., 1987) plus 100 mg/L (0.01%) or 50 mg/L (0.005%) colchicine. Colchicine was incorporated into the sterile medium by filter sterilization to avoid the possibility of heat denaturation. After this initial exposure to colchicine for either 7 or 14 days, the shoot tips were transferred to shoot-proliferation medium without colchicine for 30 days. Then the culture again was transferred to colchicine-containing medium for 14 days for a second exposure to colchicine. After the second exposure, the shoots were grown on shoot-proliferation medium until they were about 2.5 to 3.0 cm long. Then the shoots were rooted on rooting medium (medium 'D' of Singh et al., 1987) and transferred to the greenhouse. All the cultures were done in 1.5 x 14.5 cm culture tubes and incubated at 25 C with a 16 hr photoperiod under cool white fluorescent tubes in an incubator.

Flower buds undergoing meiosis were fixed and metaphase I chromosome counts were done according to Singh and Hymowitz (1985).

Results and discussion: Treating roots with 0.1 to 0.2% colchicine solution for a period of 8 to 24 hrs was reported to be effective in inducing polyploidy in various species of Gramineae (Stebbins, 1949). In this study, the application of colchicine to the axillary buds or roots did not produce plants with doubled chromosome numbers (Table 1). However, the culturing of shoot tips on colchicine-containing medium effectively induced chromosome doubling. The treatments with the higher concentration of colchicine for the shorter exposure period or lower concentration of colchicine for the longer exposure period was found to give the best results (Table 1).

Table 1. The effect of various colchicine treatments on inducing chromosome doubling in H310, a hybrid between G. latifolia x G. canescens.

Method	Treatment		No. of plants with doubled chromosomes
	Conc. of colchicine	Replications	
Axils	0.1%	5	0
Roots	0.1%	5	0
Shoot tip	0.1% 7-day [†]	4	2
	0.01% 14-day	4	0
	0.005% 7-day	4	0
	0.005% 14-day	4	2

[†]Time of initial exposure to colchicine in culture.

The second exposure to colchicine was done to ensure the chromosome doubling of unaffected sectors during the initial exposure. The putative doubled sectors (shoots) typically were slow growing with thicker stems and darker green leaves than the unaffected shoots. Care was taken to remove the occasional fast growing shoots (unaffected) during subcultures.

In addition to cytological confirmation of chromosome doubling, pod set also was observed in the amphidiploids. The mature seeds of these pods, upon germination, produced fertile plants. Thus far the chromosome number has been doubled in two additional hybrids successfully, using the shoot-tip culture method. These are H429, G. tomentella (2n=38, EE) x G. canescens (2n=40, AA) and H507, G. max (2n=40, GG) x G. clandestina (2n=40, AlAl).

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8) Biosystematics of the genus Glycine, 1988.

Table 1 contains the latest version of the species in the genus Glycine, three-letter code, $2n$ chromosome number, genome symbols, and the standards used in the determination of genomic affinities. Both the University of Illinois (IL) and the USDA (PI) numbers for each standard are presented. For authorities, please see Singh et al., 1988.

The major points are as follows:

1. Pachytene chromosome analysis validated the assigned genome symbols of GG to G. soja and G. max.
2. Glycine tomentella ($2n=78$) previously was assigned the genome symbol ?AAEE. Now it is assigned the genome symbol DDEE.
3. Glycine tomentella ($2n=80$) carries the genome symbol AADD. However, several accessions were located that possess the AA genome, but the second genome does not appear to be DD.

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Table 1. Species in the genus Glycine, three-letter code, somatic chromosome number, genome symbols, and standard accessions, 1988.

Species	Code	2n	Genome symbol	Standards	
				IL	PI
Subgenus <u>Glycine</u>					
<u>G. arenaria</u>	ARE	40	--		
<u>G. argyrea</u>	ARG	40	A2A2	768	505151
<u>G. canescens</u>	CAN	40	AA	401	440928
<u>G. clandestina</u>	CLA	40	A1A1	425	440948
<u>G. curvata</u>	CUR	40	--		
<u>G. cyrtoloba</u>	CYR	40	CC	481	440963
<u>G. falcata</u>	FAL	40	FF	674	505179
<u>G. latifolia</u>	LAT	40	B1B1	373	378709
<u>G. latrobeana</u>	LTR	40	--		
<u>G. microphylla</u>	MIC	40	BB	449	440956
<u>G. tabacina</u>	TAB	40	B2B2	370	373990
No adventitious roots		80	AAB2B2	506	440996
Adventitious roots		80	BBB2B2	640	483204
<u>G. tomentella</u>	TOM	38	EE	398	440998
		40	DD	614	446993
		78	DDEE	363	339657
		80	AADD	485	441005
		80	AA??		
Subgenus <u>Soja</u>					
<u>G. soja</u>	SOJ	40	GG		
<u>G. max</u>	MAX	40	GG		

IL numbers are temporary University of Illinois accession numbers; PI numbers are permanent numbers assigned by the USDA.

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 9) Preliminary study on the inheritance of brown stem rot resistance
PI 437685D.

Brown stem rot (BSR), caused by Phialophora gregata (Allington and Chamberlain) W. Gams, of soybean is one of the frequently occurring diseases in the midwestern USA and Canada (Athow, 1987). The fungus enters plants through the roots, causing internal stem browning as it advances upward. Severe infestations cause interveinal foliar chlorosis and necrosis often associated with yield losses ranging from 12 to 38% (Gray, 1972; Sebastian et al., 1986). All current soybean cultivars with BSR resistance have been derived from a common ancestor, Plant Introduction (PI) 84946-2. Variability in pathogenicity of P. gregata isolates collected over a four state (IA, IL, MN, WI) region has been reported (Willmot, 1988).

Three distinct dominant genes for BSR resistance have been identified. PI 84946-2 has the Rbs1 gene and possibly a minor gene for resistance (Sebastian and Nickell, 1985). PI 437833 and PI 437970 have the Rbs2 (Hanson et al., 1988) and Rbs3 (Willmot, 1988) genes, respectively. All three genes exhibit duplicate dominant epistasis with each of the other genes. New sources of resistance to BSR would be beneficial to breeding programs to develop new BSR-resistant cultivars and to combine more than one resistance gene into new genotypes to provide broader resistance to BSR. The objective of this study was to determine the nature of inheritance of BSR resistance in PI 437685D.

Materials and methods: Soybean plants were evaluated on the University of Illinois Agronomy and Plant Pathology South Farm at Urbana in 1986 and in the greenhouse in the spring of 1987 and 1988. Field and greenhouse inoculation methods were performed as described by Willmot et al. (1988). Disease ratings of field-grown plants were made on randomly selected plants from hill plots at R6 by counting the uppermost node with leaf symptoms and splitting stems lengthwise to observe stem browning. Plants were rated susceptible when the scores were within the 99% confidence interval of the leaf or stem symptom means of the susceptible cultivar 'Cumberland'. Greenhouse ratings for leaf and stem symptoms were compared to resistant (PI 437685D, PI 437833, L78-4094) and susceptible (Pioneer 9271, Century 84) standards.

Results and discussion: Greenhouse evaluation for BSR resistance provides results that are less variable than field ratings (Sebastian et al., 1986). Moisture and temperature can be easily controlled; several screening experiments can be run during fall and winter months; and

confounding symptoms from other pathogens can be reduced. Pioneer 9271 (P9271) was very susceptible (a few escapes were found) and PI 437685D, L78-4094 (Rbs1 gene from PI 84946-2), and PI 437833 were resistant to BSR in greenhouse screening (Table 1).

Field and greenhouse reactions of F2 progeny of the cross PI 437685D x P9271 fit a single dominant gene model for BSR resistance in PI 437685D (Table 2). Ratings of F2-derived F3 families (F2:3) from greenhouse evaluation revealed resistant, segregating, and susceptible families that fit a 1:2:1 model for a single dominant gene segregating in the F3 generation (Table 2).

Disease reactions of F2 progeny from PI 437685D x L78-4094 fit a 15:1 ratio for duplicate dominant epistasis (Table 3). The F2:3 families fit a 7:4:4:1 ratio for completely resistant, segregating 15:1, segregating 3:1, and completely susceptible families.

The F2 plants from the cross PI 437685D x PI 437833 also fit a 15:1 model for dominant epistasis (Table 4). The F2:3 families do not fit a 7:4:4:1 model. A few plants of PI 437833 occasionally give a susceptible reaction in greenhouse evaluation for BSR resistance (Table 1). This may make it difficult to distinguish between resistant families and families that are segregating 15:1 unless very large numbers of plants are screened. The data does fit an 11:4:1 model when combining the resistant and 15:1 segregating families.

These results reveal that resistance to BSR in PI 437685D is conferred by a single dominant gene that is apparently different from the Rbs1 and Rbs2 genes. Progeny from a cross of PI 437685D x PI 437970 will be evaluated for resistance to BSR to determine whether the gene in PI 437685D is different from the Rbs3 gene present in PI 437970.

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_____, C.D. Nickell and R.L. Nelson. 1988. Preliminary study on the inheritance of brown stem rot resistance in PI 86150 and PI 423930A. Soybean Genet. Newsl. 15:103-106.

Table 1. Reaction of parental lines to inoculation with P. gregata in the greenhouse.

Parents	Resistant	Susceptible
	-----Number of plants-----	
PI 437685D	45	0
L78-4094	35	0
PI 437833	43	2
P9271	3	38

Table 2. Reaction of F2 plants and F2:3 families of the cross PI 437685D x P9271 to inoculation with P. gregata.

Gener- ation	Test site*	Plants or families+			Ratio tested	χ^2	P
		R	H	S			
		--No. of plants--					
F2	Fld.	36		10	3:1	0.26	0.50-0.75
	G.H.	49		11	3:1	1.42	0.25-0.50
	Pooled	85		21	3:1	1.52	0.10-0.25
	Contingency					0.02	0.75-0.90
Families							
F2:3	G.H.	7	11	6	1:2:1	0.25	0.75-0.90

*Fld.=field in 1986; G.H.=greenhouse in 1987 for F2 and 1988 for F2:3.

+Plants and families were classified as resistant (R), heterozygous (H), or susceptible (S).

Table 3. Reaction of F2 plants and F2:3 families of the cross PI 437865D x L78-4094 to inoculation with P. gregata.

Gener- ation	Test site*	Plants or families+				Ratio tested	χ^2	P
		R	H	S				
		---No. of plants---						
F2	Fld.	40		4	15:1	0.61	0.25-0.50	
	G.H.	93		7	15:1	0.10	0.75-0.90	
	Pooled	133		11	15:1	0.47	0.25-0.50	
	Contingency					0.01	0.90-0.95	
Families								
F2:3	G.H.	16	12	11	1	7:4:4:1	1:53	0.50-0.75
		28	11	1		11:4:1	1:01	0.50-0.75

*Fld.=field in 1988; G.H.=greenhouse in 1987 for F2 and 1988 for F2:3.

+Plants and families were classified as resistant (R), heterozygous (H), or susceptible (S).

Table 4. Reaction of F2 plants and F2:3 families of the cross PI 437865D x PI 437833 to inoculation with P. gregata.

Gener- ation	Test site*	Plants or families+			Ratio tested	χ^2	P	
		R	H	S				
		---No. of plants---						
F2	Fld.	36		3	15:1	0.01	0.90-0.95	
	G.H.	94		6	15:1	0.01	0.90-0.95	
	Pooled	130		9	15:1	0.01	0.90-0.95	
	Contingency					0.00	0.99-1.00	
Families								
F2:3	G.H.	11	21	7	1	7:4:4:1	16.31	<0.005
		32	7		1	11:4:1	2.54	0.25-0.50

*Fld.=field in 1988; G.H.-greenhouse in 1987 for F2 and 1988 for F2:3.

+Plants and families were classified as resistant (R), heterozygous (H), or susceptible (S).

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 1) Studies of protein modification during initiation of somatic embryogenesis in the soybean.

Development of tissue culture systems has made it possible to regenerate soybean plants from culture by way of somatic embryogenesis. The explant source is an immature cotyledon (Ranch et al., 1985). The early stages of initiation of the process of embryogenesis represent a significant switch in developmental pathways, i.e., a somatic cell destined for storage functions becomes embryogenic. Studies to resolve the mechanisms underlying initiation of the embryogenic response have not been reported for soybean.

Histological analysis of cotyledonary tissue indicates that embryogenic foci are made up of cytoplasmically dense cells located only a few cell layers beneath the surface and that only a very small percentage of cells within the cotyledon respond to the initiation treatment. The low ratio of responding cells to non-responding cells make it difficult to detect subtle changes in protein components of responding tissue relative to non-responding tissue. The mechanism by which cells embark on a pathway of embryogenesis is not known. Probably, the induction treatment induces a de novo protein involved in this developmental trigger or the treatment induces a post-translational modification of an existing protein which then serves a role in the developmental 'switch'. Post-translational modification of proteins has long been recognized as an important control phenomenon in the regulation of cell division, genome activity, protein synthesis, and membrane permeability (Trewavas, 1976). Our objectives were to evaluate the effectiveness of protein gel electrophoresis in detecting early changes in protein modification (phosphorylation) during the initiation of somatic embryogenesis in soybean.

Materials and methods: Tissue culture and embryo induction: Immature cotyledons (4 mm) of 'Williams 82' were harvested from greenhouse-grown plants. Pods were sterilized and embryos excized and placed on various media. Media used included MS without auxin, MS with 20 mg/L 2,4-D, MS with 10 mg/L NAA, or MS with 20 mg/L IAA. At various times post-initiation (0, 1, 3, 5, 7, and 11 days) cotyledons were removed from the initiation media and placed on an identical medium containing ³²P (ortho-phosphate) at a concentration of 0.1 mCi/100 ml medium. Explants were left on the radioactive medium for 24 hours.

Protein extraction and gel electrophoresis: Explants were ground-up in an extraction buffer (10 mM Tris-HCl, 0.1% Mercaptoethanol, 2.0% SDS, pH 7.8). The crude homogenate was cleared by micro-centrifuging at 15,000 g for 5 minutes. The protein located in the resulting supernatant was quantitated by a

modification of the Folin assay (Peterson, 1977). Proteins were separated in SDS-polyacrylamide gels (12.5%) with a 5% acrylamide stacking gel at 40 mA for 5 hours. Lanes were loaded with equivalent amounts of total protein (50 - 75 ug)

Protein detection: Total protein was visualized by staining gels in 0.1% Coomassie Brilliant Blue made up in a solution of 45% methanol and 9.5% glacial acetic acid. Gels were destained overnight in 20% methanol and 9.5% glacial acetic acid solution. Gels used for detection of phosphorylated proteins were placed immediately into a solution of 55% methanol and 2% glycerol overnight. Gels were dried beneath acetate sheets at about 70 °C under vacuum on a GSD-4 Slab gel-drier (Pharmacia). Radio-labelled proteins were visualized by exposure to Kodak X-Omat AR Diagnostic film with a DuPont Cronex Lightning-Plus Intensifying screen at -70 °C.

Results: Auxins are known to induce rapid changes in gene expression in plant tissues (Key et al., 1986; Theologis, 1986). Therefore, it is necessary to distinguish, if possible, those genetic responses unique to somatic embryo induction, versus those responses common to auxin gene regulation. For this reason 3 different auxins were tested as well as a negative control (0 auxin). In all instances, with or without auxin treatment, explants that had been in culture for 3-5 days incorporated more ³²P per ug total protein than explants freshly placed in culture (0 or 1 day) or that had been in culture longer (7 or 11 days). Many bands representing phosphorylated proteins were observed on autoradiograms. Many of these proteins appeared to be constitutively expressed and/or phosphorylated. These proteins were observed under all conditions tested (0 auxin, IAA, NAA, or 2,4-D) and at all time points. Relatively intense bands were observed which corresponded to protein of approximately 11 KD, 20-22 KD, 31 KD, 40 KD and 97 KD.

Explants plated on MS media without auxin produced an approximate 25 KD phosphorylated protein beginning 5 days after being placed in culture. This protein also was faintly visible in protein extracts from explants placed on media containing IAA, but was not seen in explants placed on media containing NAA or 2,4-D. It is interesting to note that both NAA and 2,4-D will induce somatic embryogenesis under our conditions, whereas MS media without auxin or IAA, will not. All auxin-treated explants, at all time points, produced a low molecular weight phosphorylated protein of about 12 KD. This protein was not observed to be phosphorylated in explants placed on media without auxin.

These preliminary results suggest that differences in protein modification can be detected between auxin and '0' auxin treatments. This result is expected. More importantly, even using relatively insensitive 1-D SDS-PAGE, differences are apparent between auxin treatments. These differences may provide a means to evaluate the effectiveness of various treatments in induction of somatic embryogenesis in soybean and to identify those unique responses associated with induction.

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 2) The third mutation derived from the cultivar Beeson.

In a commercial field of cv. 'Beeson', Dr. Marc Albertsen isolated three independent male-sterile mutations. Two of them have been assigned to known loci: a male-sterile, female-fertile mutation was located at the ms1 locus, and a male-sterile, female-sterile mutation at the st5 locus (Skorupska and Palmer, 1988, 1989). The third mutation is characterized by plants that set a few pods and was classified as a male-sterile, female-fertile mutation. Our temporary designation for this mutation is Beeson-ms.

Cytological tests (anther staining in I2KI) showed a variety of abnormalities in microspore development. Detailed observations conducted on anthers revealed variation in the number of degenerated anthers from flower to flower, even from the same plant. In some plants one flower produced normal pollen, the other had highly degenerated anthers. In some flowers the appearance of the upper whorl of stamens looked normal whereas the stamens of the bottom whorl had brownish anthers. There was wide variation in the number of normal anthers in a flower. Moreover, within an anther, locules containing degenerated microspores were observed, and adjacent locules had well stained pollen. No regularity was found in the number of locules with well stained pollen and locules with degenerated microspores. Three degenerated locules and one normal were observed in some anthers and three normal and one degenerated locules were seen in anthers from the same flower. A characteristic feature of this mutant was

the formation of aggregates containing all the contents of the locule. Individual well stained microspores were visible but were not separated from each other. During cytological preparation, it was observed that microspores released from the locule still preserved the elongated shape of the locule. The variation in abnormalities caused by this mutation indicated that microspore development was affected during several different stages.

Allelism tests were conducted with ms1, ms2, ms3, ms4 and ms6 mutants. In the F1 generation of testcrosses only fertile plants were observed. The pattern of segregation for F2 families fit the expected 1:1 ratio (Table 1). The F2 families segregated for sterility in the 3:1 or 9:7 ratio. Within F2 families, segregation for both ratios was very clear and a large number of plants were observed (Tables 2,3,4,5). Results showed that the third mutation found in Beeson is not allelic to the ms1, ms2, ms3, ms4 and ms6 loci. Allelism tests will be conducted with the ms5 mutant and with the partial male sterile mutant (msh).

Linkage tests were conducted with the male-sterile Beeson mutation and the T locus (tawny pubescence). Linkage values were calculated by using the Linkage-1 program which utilizes the maximum likelihood method (Suiter et al., 1983). In the F2 generation, segregation for male sterility and pubescence color showed independent assortment of observed loci (Table 7).

Table 1. Segregation of F2 families from crosses of Beeson-ms with the ms1, ms2, ms3, ms4, and ms6 mutants.

Cross combination	F2 (3:1)		F2 (9:7)		Total	χ^2	P>
	Obser.	Exp.	Obser.	Exp.			
<u>ms1</u> <u>ms1</u> x Beeson Ms ms	3	2.5	2	2.5	5		
Beeson-ms x <u>Ms2</u> <u>ms2</u>	40	36	32	36	72	0.88	0.25
Beeson-ms x <u>Ms3</u> <u>ms3</u>	29	27	25	27	54	0.29	0.50
Beeson-ms x <u>Ms4</u> <u>ms4</u>	15	13.5	12	13.5	27	0.33	0.50
<u>ms6</u> <u>ms6</u> x Beeson Ms ms	16	17	18	17	34	0.11	0.50

Table 2. Segregation in the F2 population of the cross ms1 ms1 (T266) x Beeson Ms ms

	Ratio					
	3:1			9:7		
	Fertile	Sterile	Total	Fertile	Sterile	Total
Observed	260	74	334	140	100	240
Expected	250.5	83.5	334	135	105	240
χ^2 pooled (1 df)		1.45	P>0.10	(1 df)	0.43	P>0.75
χ^2 total (3 df)		2.47	P>0.25	(2 df)	1.75	P>0.25
χ^2 homogeneity (2 df)		1.02	P>0.25	(1 df)	1.32	P>0.25

Table 3. Segregation in the F2 population of the cross Beeson ms ms x Ms2 ms2 (T253H)

	Ratio					
	3:1			9:7		
	Fertile	Sterile	Total	Fertile	Sterile	Total
Observed	2926	941	3867	1988	1655	3643
Expected	2900.25	966.75	3867	2049.2	1593.8	3643
χ^2 pooled (1 df)		0.91	P>0.25	(1 df)	4.17	P>0.02
χ^2 total (40 df)		33.68	P>0.50	(32 df)	17.80	P>0.95
χ^2 homogeneity (39 df)		32.77	P>0.75	(31 df)	13.63	P>0.99

Table 4. Segregation in the F2 population of the cross Beeson msms x Ms3 ms3 (T291H)

	Ratio					
	3:1			9:7		
	Fertile	Sterile	Total	Fertile	Sterile	Total
Observed	2257	714	2971	1464	1232	2696
Expected	2228.25	742.75	2971	1516.5	1179.5	2696
χ^2 pooled (1 df)		1.48	P>0.10	(1 df)	4.15	P>0.02
χ^2 total (29 df)		13.92	P>0.99	(25 df)	13.09	P>0.97
χ^2 homogeneity (28 df)		12.43	P>0.99	(24 df)	8.94	P>0.99

Table 5. Segregation in the F2 population of the cross Beeson ms ms x Ms4 ms4 (T292H)

	Ratio					
	3:1			9:7		
	<u>Fertile</u>	<u>Sterile</u>	<u>Total</u>	<u>Fertile</u>	<u>Sterile</u>	<u>Total</u>
Observed	1268	418	1686	763	681	1444
Expected	1264.5	421.5	1686	812.25	631.75	1444
χ^2 pooled (1 df)	0.12	P>0.50		(1 df) 6.82	P>0.005	
χ^2 total (15 df)	1.23	P>0.99		(12 df) 8.12	P>0.75	
χ^2 homogeneity (14 df)	1.10	P>0.99		(11 df) 1.29	P>0.99	

Table 6. Segregation in the F2 population of the cross ms6 ms6 (T295H) x Beeson Ms ms

	Ratio					
	3:1			9:7		
	<u>Fertile</u>	<u>Sterile</u>	<u>Total</u>	<u>Fertile</u>	<u>Sterile</u>	<u>Total</u>
Observed	1182	380	1562	1083	897	1980
Expected	1171.5	390.5	1562	1113.75	866.25	1980
χ^2 pooled (1 df)	0.37	P>0.50		(1 df) 1.94	P>0.10	
χ^2 total (16 df)	3.25	P>0.99		(18 df) 3.33	P>0.99	
χ^2 homogeneity (15 df)	2.87	P>0.99		(17 df) 1.39	P>0.99	

Table 7. Linkage tests between Beeson-ms mutation and the T locus

Cross combination		Phenotype frequency				
		<u>a</u>	<u>b</u>	<u>c</u>	<u>d</u>	<u>Total</u>
<u>TT</u> <u>ms</u> <u>ms</u> Beeson x						
<u>tt</u> <u>Ms6</u> <u>Ms6</u> (T295)	Observed	882	282	264	80	1508
	Expected	848.2	282.8	282.8	94.25	1508

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3) Allelism tests of a tissue-culture derived male-sterile, female-sterile soybean mutant.

Graybosch et al. (1987) reported recessive mutations for putative sterility characters in cultivars 'Funman' and 'Calland' from tissue-culture derived plants. Plants regenerated from culture (Ro generation) were allowed to self-pollinate and were threshed individually. Progeny of Ro plants (R1 generation) were grown in the field. Little variability in qualitative traits was observed among the approximately 7000 R1 plants. At maturity, all replications of Calland-009 contained sterile plants. Segregation ratios for the sterility character(s) fit a 3:1 (fertile : sterile) ratio expected if a recessive mutation had occurred in an Ro plant (Graybosch et al., 1987).

This report describes the genetics of the Calland mutation and includes allelism tests with the st2, st3, and st4 mutations. Allelism tests with the st5 mutation are not complete.

Methods: Allelism tests of the Calland sterile were conducted with three nonallelic asynaptic or desynaptic mutants, st2 (T241), st3 (T242), and st4 (T258) by crossing known heterozygotes. The heterozygous genotype of the parental plants was determined by progeny testing following self-pollination. This permitted us to distinguish the AA genotype from the Aa genotype.

If two lines were allelic with regard to their sterility, then one out of four F1 plants would be sterile. In the F2 generation, nonsegregating families and families segregating 3 fertile : 1 sterile plants would be observed. If different genes were controlling sterility in the two lines, no sterile plants would be observed in the F1 generation. Moreover, the F2 generation would include nonsegregating families, families segregating 3 fertile : 1 sterile plant, and families segregating 9 fertile : 7 sterile plants.

Results: No sterile F1 plants were observed in any of the three different allelism testcrosses. The F2 ratio of nonsegregating families to 3:1-segregating families to 9:7-segregating families fit the expected 1:2:1 ratio for nonallelism in all cross combinations (Table 1).

The F2 family data for the 3:1 and 9:7 segregations are presented in Tables 2, 3, and 4. Within each cross combination, the data are homogeneous either for the 3:1 or 9:7 segregation.

Linkage tests were conducted with the tissue-derived male-sterile mutant and the w1 locus. Calculations were based on the 'Linkage-1' program of Suiter et al. (1989). No linkage was found between the analyzed loci (Table 5).

These data indicate that a different gene in Calland is involved in male sterility, female sterility than in T241 (st2), T242 (st3), and T258 (st4).

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Table 1. Number of F2 families that were all fertile plants 3:1 segregation, or 9:7 segregation from crosses between known heterozygous parent plants.

Cross	F2 generation: number of families							P
	All fertile	3:1	9:7	Expected ratio			$\chi^2(1:2:1)$	
Calland het. x <u>St2 st2</u>	6	10	7	5.75	11.5	5.75	0.48	>0.75
Calland het. x <u>St3 st3</u>	5	14	6	6.25	13	6.25	0.34	>0.75
Calland het. x <u>St4 st4</u>	4	9	6	4.75	9.5	4.75	0.47	>0.75

Table 2. Probability for expected ratios in segregating F2 families from crosses between Calland heterozygous plants with St2 st2.

	<u>No. of plants</u>		df	$\chi^2(3:1)$	P	<u>No. of plants</u>		df	$\chi^2(9:7)$	P
	Fert.	Ster.				Fert.	Ster.			
	1038	343				514	402			
Total			10	2.16	>0.99			7	2.11	>0.95
Pooled χ^2			1	0.02	>0.99			1	0.01	>0.99
Homogeneity χ^2			9	2.14	>0.97			6	2.10	>0.90

Table 3. Probability for expected ratios in segregating F2 families from crosses between Calland heterozygous plants with St3 st3.

	No. of plants		df	$\chi^2(3:1)$	P	No. of plants		df	$\chi^2(9:7)$	P
	Fert.	Ster.				Fert.	Ster.			
	2232	715				700	526			
Total			14	5.73	>0.95			6	0.83	>0.99
Pooled χ^2			1	0.86	>0.25			1	0.36	>0.50
Homogeneity χ^2			13	4.87	>0.97			5	0.47	>0.99

Table 4. Probability for expected ratios in segregating F2 families from crosses between Calland heterozygous plants with St4 st4.

	No. of plants		df	$\chi^2(3:1)$	P	No. of plants		df	$\chi^2(9:7)$	P
	Fert.	Ster.				Fert.	Ster.			
	1322	439				477	371			
Total			9	0.005	>0.99			6	0.34	>0.99
Pooled χ^2			1	3.05	>0.005			1	0.00	
Homogeneity χ^2			8	3.045	>0.90			5	0.34	>0.99

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4) Apetally mutant in soybean)

Origin

In the F12 generation, one progeny row of the cross SRF 200 x (SRF 350 x Tracy) segregated 7 fertile to 9 almost completely sterile plants. Progeny tests of the seven fertile plants were conducted in F13 generation. Four progenies segregated in a 3:1 ratio; two progenies did not segregate, and in one progeny more sterile than fertile plants were observed (Table 1).

Table 1. Sterility segregation in F13 families from cross SRF200 x (SRF 350 x Tracy)

Progeny	<u>Number of plants</u>		χ^2 (3:1)	P >
	Fertiles	Steriles		
1	95	29	0.17	0.50
2	32	40	35.85	
3		NS*		
4	85	28	0.003	0.97
5		NS		
6	111	35	0.08	0.75
7	105	29	0.81	0.25

*-Nonsegregating for sterility

A few seeds were harvested from sterile plants in progeny number two. They produced sterile plants with abnormal flower buds. Seeds from these mostly sterile plants were harvested again and planted in the field three times at two-week intervals. No fertile plants were observed in the progeny. All plants were sterile and had abnormal flowers.

Characteristic features of analyzed mutant are:

- Lack of banner petal, lack of lateral wings, and lack of a keel petal. (Occasionally a keel petal was observed)
- Abnormal pattern in the development of androecium; number of anthers was greatly reduced. In 30 flowers (10 plants x 3 flowers per plant) forty percent of the flowers had two stamens, a complete complement of

stamens was observed in only two flowers. Reduction in the length of filaments was noticed; some of them were shorter than in normal flowers and therefore unable to approach the stigma

- Style was curved acutely towards the pistil, causing difficulties in performing cross pollination
- The abnormal staminal tube was surrounded by calyx, eliminating the possibility of outcrosses.
- Microspores had collapsed cytoplasm; individual pollen grains stained normally with I2KI

Cleistogamous flowers and lack of fertile plants in the progeny of sterile plants suggests that some fertility exists in this apetally mutant, allowing reproduction. Seed set was analyzed. The apetally mutant produced an average of 3.9 seeds per plant (Table 2).

Table 2. Seed set of apetally mutant in soybean

Planting	Number of			Seed/plant	Seed/pod
	Plants	Pods	Seeds		
I	44	140	173	3.9	1.2
II	38	104	128	3.7	1.2
III	19	70	89	4.7	1.3
Total	101	314	390	3.9	1.2

The apetally mutant can be classified as a structural flower mutant. Additional genetic markers of this mutant are sterility (st?, ms?) and narrow leaves (ln). Structure of the flowers can lower efficiency of crosses, and female fertility must be defined before this mutant can be used effectively in genetic mapping studies.

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 5) Efficiency and phage sensitivity of *Bradyrhizobium japonicum* serogroup 123 isolates native to Iowa soils,

Bradyrhizobium japonicum serogroup 123 has been the focus of much research in recent years due to its dominance in the nodules of soybean grown in Midwestern soils. Members of this highly competitive serogroup normally occupy 60-80% of soybean root nodules in Iowa (Damirigi et al., 1967; Berg et al., 1988). However, serogroup 123 strains, as characterized by USDA 123, have been shown to be inferior in N₂ fixation relative to USDA 110 (Ham, 1980).

The inability to successfully replace the indigenous strains of *B. japonicum* serogroup 123 by inoculum application of more efficient strains suggests that factors other than bacterial numbers play important roles in their competitiveness. During 1983 and 1984, Berg et al. isolated over 100 strains of *B. japonicum* belonging to serogroup 123 from Iowa soils. These isolates were characterized for their growth characteristics and colony morphologies. Hickey et al. (1987) showed by using SDS-PAGE that considerable diversity existed among the 123 isolates in their total protein composition. In current studies, we are further characterizing these isolates based on their greenhouse efficiency, bacteriophage sensitivity, competitiveness, and total genomic analysis.

Thirty-nine bacterial strains from Berg's collection were randomly chosen and 20 rhizophages were isolated against them. For phage isolation, six soils from six Iowa counties were collected and mixed together. Thirty-nine 10-g samples of this composite soil were enriched by separate additions of 100 ml of each bacterial culture, which had been grown in flasks to a common optical density of 1.0 (10^8 - 10^{10} cells/ml). These were incubated for 18-22 h, centrifuged, and the supernatant passed through a 0.22-um membrane filter. Aliquots of the filtered supernatant were then added to other flasks containing the same bacterial strain at the same density. Twenty phage cultures were obtained as clearing lysates and purified by repeating the serial transfer several times. The cultures received five drops of chloroform and were kept at 4 C (Somasegaran, 1985).

Phage sensitivity patterns were recorded when each bacterial strain was typed against all phage isolates (Table 1). Reactivity of bacterial strains with phage cultures occurred in eight distinct patterns ranging from zero to eight reactions in each group.

A greenhouse experiment was also conducted to test the efficiency of the 39 bacterial strains under sterilized conditions. Three-day-old pregerminated soybean seedlings ('Williams' 82) were inoculated with bacterial strains at O.D. = 1.0, and pots were replicated four times in a completely randomized design. Strains USDA 110 and USDA 123 were used as controls, and 12 pots did not receive any inoculum. Six-week-old plants (V-6 stage) were harvested and total plant dry weights were measured. Data were analyzed by LSD and a histogram of means of all treatments was made (Graph 1). Significant differences were observed among the

bacterial isolates based on plant total dry weight. Some of the tested strains were found to be as efficient as USDA 110 (Treatment #41). USDA 123 (Treatment #40) did not seem to be representative of the tested isolates and plants inoculated with this strain were significantly lower in plant dry weight when compared with some of the other 123 isolates.

We are also conducting competition studies to compare the competitiveness of these isolates against *B. japonicum* strains USDA 110 and USDA 123. We are especially interested in those strains of serogroup 123 shown to be comparable to USDA 110 in N₂-fixing ability.

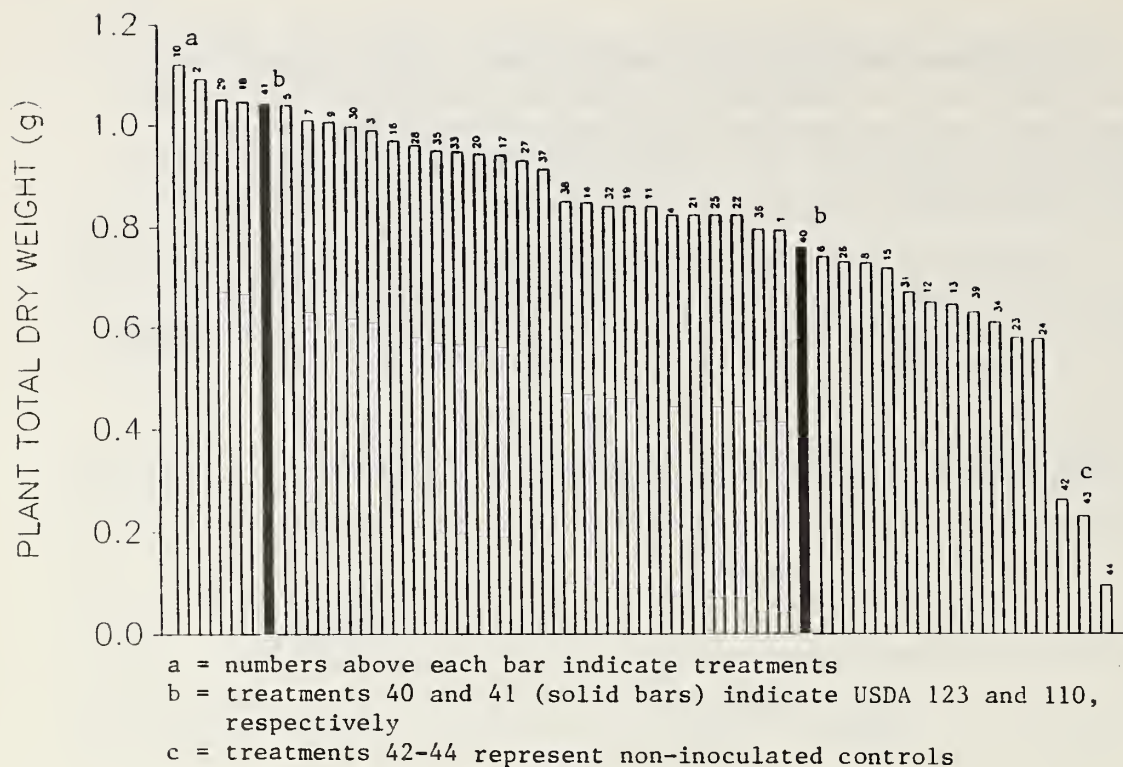
Table 1. Phage-typing patterns of *B. japonicum* 123 isolates

Bacterial strains	1								Positive reactions
	Phages								
	2								
Type of Reaction									
S3-5	+	+	+	+	*	+	*	*	8
G6-1	+	+	+	+	+	+	0	0	6
S1-1	+	+	+	+	0	+	0	0	5
G6-6	+	0	0	+	+	0	0	0	3
G6-7	0	0	0	0	+	0	0	+	2
H1-9	0	0	0	0	0	0	0	+	1
G3-2	0	0	0	0	0	0	0	0	0

- 1 - Phages are identified by the bacterial strain from which it was first isolated.
- 2 - 0 = no phage formation; + = confluent growth; * = lysogenic reaction

Total genomic analysis of all 39 bacterial isolates is underway as a further means to divide these strains into more stringent groupings. These groups will be based on individual restriction fragment length comparisons. A modified miniprep DNA isolation procedure (Maniatis et al., 1982) is being used to extract bacterial DNA. The DNA is digested using various endonucleases, and resulting fragments are separated with agarose gel electrophoresis.

The results of the genomic analysis will be compared with the unique phage sensitivity patterns, competitiveness, and efficiency data, enabling us to distinguish and possibly isolate a particular strain of interest within the diverse pool of serogroup 123 strains.



Graph 1. Histogram of efficiency data from 39 serogroup 123 isolates and USDA 110 and 123, based on plant total dry weight.

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6). Chloroplast abnormalities in three chlorophyll-deficient mutants from the w4-mutable line of soybean.

Mutagenesis experiments conducted in our laboratory with the transposable element-containing line, w4-mutable, have yielded many mutants (Groose and Palmer, 1987). Eight are chlorophyll-deficient mutants. Isozyme analysis of three of these, CD1, CD2, and CD3 (Groose et al., 1987) showed loss of two of the three bands for mitochondrial malate dehydrogenase (MDH) (Hedges, 1989). Genetic tests show the three variants to be allelic. It is suggested that they may each represent different size deletions, induced by the action of a transposable element after its excision from the w4 locus (Hedges, 1989).

Chloroplast structure and chlorophyll content of CD1, CD2, and CD3 are being studied. As control plants we are using: a 'Harosoy' w4 isoline; w4-mutable plants whose purple-flecked green hypocotyls indicated the presence of an active transposable element; and y9, a yellow mutant unrelated to the w4-mutable line.

Chloroplasts of unifoliate leaves collected from CD1, CD2, and CD3 plants at 18 days postplanting have irregular chloroplasts that contain no starch grains. Grana are rudimentary or absent. Unifoliate leaves from green control plants had chloroplasts with abundant starch grains and well stacked grana. Most notable in the chlorophyll-deficient plants is the prevalent arrangement of the grana into whorls. These structures are present in CD1, CD2, and CD3 unifoliate leaves collected at 18 days postplanting, and in lesser amounts, in the trifoliate leaves collected at 27 days postplanting.

Chloroplasts from trifoliate leaves of the mutant plants contain fewer starch grains than do normal green plants. Increased grana stacking and lessened grana distortion was observed. CD3 plants appear to normalize earlier in development than do CD1 or CD2 plants. This is reflected in the greater seedling vigor, and faster greening-up of CD3 plants in the field. The y9y9 plants exhibit abnormalities very different from those seen in CD1, CD2, and CD3. Chlorophyll extraction studies are being conducted. Absorption spectra support the similarities of CD1, CD2, CD3, and the differences between y9 and CD1, CD2, CD3. All CD plants and y9 plants have absorption spectra differing from those of normal green plants.

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 7) Characterization of three necrotic root mutants,|

Three necrotic root mutants (NR1, NR2, NR3) were recovered from mutagenesis experiments with w4-mutable. These mutants occurred as separate events (Blomgren et al., 1987). Necrosis is first seen in the area of root cell elongation as brown sectors of inwardly collapsed cells. These sectors increase in number and size until the entire root is necrotic. The meristematic areas remain normal on the main root and the side roots.

Light microscopy study of the roots is in progress. Preliminary results reveal that necrotic roots have degenerating epidermal and cortical cells. This suggests that an outer layer of dead, nonfluorescing cells obscures fluorescing layers. However, large numbers of bacteria were observed in the microscopy sections. These bacteria surround the root and penetrate into the cortex. Penetration is between, and not through, cells. It is possible that the outer obscuring layer could be a substance produced by the bacteria. We wanted to know whether the necrosis was occurring as a reaction to the bacteria (i.e., a plant loss of resistance), or whether the bacteria multiplied as an opportunistic infection of already degenerating tissue.

An experiment was designed to grow plants in a sterile environment. Known homozygous recessive seed of NR1, NR2, NR3, known homozygous dominant seed of NR1, NR2, NR3, and seed of a 'Harosoy'-w4 isolate were used. Eight different treatments were used (Table 1). Seed sterilization was 5 minutes in 10% Clorox with a drop of detergent, rinse in autoclaved distilled water; wash for 2 minutes in 70% ETOH, and three rinses in fresh 70% ETOH. Germination paper was sterilized by autoclaving for 20 minutes; Seal-a-meal packs were autoclaved 15 minutes and either left open or heat-sealed. All work was done under a laminar flow hood. Four replications were made. The pouches were taped onto racks in a growth chamber divided into four sectors. Within each sector the pouches were placed in a complete randomized block design. Unsealed pouches were watered with autoclaved water as needed. After 11 days the pouches were opened under a laminar flow hood and scored. In all cases, known homozygous recessive plants had necrotic roots with typical lesions, although some necrotic roots seemed to be less 'slimy' (Table 2). Samples were collected for microscopy. Necrotic roots grown under sterile conditions showed the same type of cellular degeneration, but a much lower visible incidence of bacteria.

Necrotic root plants are lethal in the field. However, in the greenhouse, these plants are weak but may yield one seed per plant. For genetic analyses, grafting of known necrotic root scions onto known homozygous dominant necrotic root stock was done. Linkage tests to CD1, CD2, CD3, y20-k2, k2, Harosoy-w4, and w4-mutable are being conducted. At the present time, only linkage analysis of Harosoy-w4 with NR1, NR2 and NR3 is complete. NR1, NR2, and NR3 were found to segregate independently of the w4 locus (data not presented).

Table 1. Sterility treatments used for germination of necrotic and normal plants

Treatment	Sterile seed	Sterile germination paper	Sealed pack
1	+	+	+
2	+	+	-
3	+	-	-
4	+	-	+
5	-	+	+
6	-	+	-
7	-	-	-
8	-	-	+

Table 2. Results of experiment to grow necrotic and normal plants under sterile conditions[^]

Sample*	Necrotic root	Normal root	No germination
A1**	11	0	1
A2	11	0	1
A3	10	0	2
A4	11	0	1
A5	3	0	9
A6	10	0	2
A7	9	0	3
A8	7	0	5
B1-B8	0	64	32
C1-C8	0	67	29

[^](A = known homozygous recessive for necrosis; B = known homozygous dominant for necrosis; C = Harosoy-w₄ control)

*Summation of all four replications

**A1 = homozygous recessive necrotic root seed sterilized, placed on autoclaved paper, and in sealed pouch. See Table 1 for description of treatments 1 - 8.

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Blomgren, S.M., R.W. Groose and R.G. Palmer. 1987. Necrotic root mutants in a genetically unstable line of soybean. Soybean Genet. News1. 14:171-173.

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8) Genetic test for apomixis in the msl mutant of soybean.

Introduction: The nuclear male-sterile, female-fertile msl gene in soybean (Glycine max [L.] Merr.) is inherited monogenically. Seven independent spontaneous mutations at the msl locus have been identified. (Skorupska and Palmer, 1988). The msl locus is associated with reduced female fertility and increased frequencies of polyembryony, polyploidy, and haploidy in the progeny of sterile plants (Chen et al., 1985; Kennell and Horner, 1985). Previous studies indicated that the msl gene causes abnormal development of the embryo sac which leads to mature megagametophytes with up to four times the normal number of nuclei (8 to 32) and that the msl msl plants occasionally produce seeds in the greenhouse where pollinating vectors are assumed to be absent (Chen et al., 1987; Cutter, 1975; Cutter and Bingham, 1977; Kennell and Horner, 1985). In this study, we tried to address the question how these seeds originated in the absence of out-crosses. The only sources would be self-pollination with functional pollen from msl msl plants, or apomixis. Our goal was to test whether or not any type of apomixis occurs in msl msl soybean plants. If apomictic reproduction does occur, it may simplify hybrid seed production in soybean and increase opportunities for developing superior genetic combinations.

Materials and methods: The material for the genetic tests was from crosses between the Urbana male-sterile msl line (T266H) and a white flowered chlorophyll-deficient line. The chlorophyll-deficient mutation was from T219H, and the flower color mutant, recessive allele wl, was derived from previous crosses. The chlorophyll-deficient gene yll is inherited codominantly; 1 green (Y11Y11) : 2 yellow-green (Y11yll) : 1 yellow lethal (yllyll). Flower color mutant gene wl is inherited recessively and segregates 3 purple (Wl -) : 1 white (wlw1) in F2 populations.

In our experiment, yll and wl loci were used as genetic markers. Crossing was between mslmslY11Y11W1W1 and MslMslY11yllwlwl plants. In the F1 population, all green plants (Msl - Y11Y11W1-) were discarded and all yellow-green plants (Msl - Y11yllW1-) were saved to generate F2 populations. The F2 plants were grown in the Agronomy greenhouse in Ames, Iowa. Fertility, plant color and flower color were checked. All fertile, green, purple flower plants, i.e., Msl - Y11Y11W1-, were discarded. Yellow lethal plants died at young seedling stage. The remaining plants were male-sterile, yellow-green, and white flower (mslmslY11yllwlwl) and became the material for genetic test for apomixis. Flowers on the male-sterile, yellow-green, white-flower plants were emasculated. If any seed would be produced by these emasculated flowers, chromosome number and segregation ratio of plant color were checked in the progeny. All plants should be white flower.

Results and discussion: A total of 5929 emasculations were made during summers of 1987 and 1988 in the Agronomy greenhouse at Ames, Iowa. No seed were found on plants on which flowers were emasculated.

Therefore, there was no evidence from this experiment to support the occurrence of apomixis in msl msl soybean plants. A few seed were produced by flowers that were not emasculated. This experiment suggests that the presence of progeny and variation in chromosome number of progeny in the msl msl mutant may be explained by fertilization between egg and a functional coenocytic pollen grain.

Another possibility is that apomixis does occur in msl msl plants, but it occurs only when the stigma receives a signal produced by the interaction between stigma and pollen grains. This signal is transferred into the ovule to stimulate apomictic reproduction (Lersten, 1980). When flowers are emasculated, coenocytic pollen grains are not present, therefore, apomixis cannot be induced.

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9) Genetic analyses of hybrid progeny from crossing msl msl plants and tetraploid cultivars

Introduction: In some previous work, attempts to obtain triploid soybean plants ($2n=3x=60$ chromosomes) through natural cross-pollination and artificial cross-pollination between autotetraploids and diploids were unsuccessful (Porter and Weiss, 1948; Sadanaga and Grindeland, 1981).

The reason for the absence of triploid progeny from those crosses might be related to unsuccessful endosperm development. According to the hypotheses of endosperm development, female-to-male genome in endosperm, or endosperm balance number (EBN) of female to EBN of male in endosperm must be 2:1 to give rise to viable embryos (Nishiyama and Inomata, 1966; Johnston et al., 1980; respectively). Triploids cannot be produced by crossing a diploid with a tetraploid soybean plant because the maternal:paternal genome or EBN ratio will be either 4:3 or 1:1 in reciprocal crosses, respectively. However, when a tetraploid is used as male and the msl mutation is used as a female, a tetraploid may be produced if diploid eggs function. The objectives of these experiments are 1) to examine if tetraploid progeny can be produced by crossing diploid msl msl plants with tetraploid cultivars; 2) to investigate the origin(s) of diploid eggs.

Materials and methods: The female parent used in this cross (mslmslYllyllwll) was the progeny of the Urbana male sterile msl (T266H) line crossed with a white flower, yellow-green plant color line. Male parents were tetraploids from cultivars 'Lincoln', 'Clark', 'Dunn', and 'Dunfield'. They were all fertile green plants (MslMslMslMslYllyllYllyll). This experiment was conducted in summers of 1987 and 1988 in the Agronomy greenhouse at Ames, Iowa. Crosses were made between diploid msl msl plants as female and tetraploid plants as male parent. Seeds (if any) from crosses were collected. Chromosome number, plant color, and fertility were checked in the F1 population.

Results and discussion: A total of 2007 crosses were made during summers of 1987 and 1988. A total of 30 F1 seeds were obtained (Table 1). All were fertile with 80 chromosomes (tetraploid). No triploids were found.

Table 1. F1 progeny from msl msl plant X tetraploid

Crosses	F1 plant color and number of seeds		
	Green* (<u>YYYY</u>)	Green-yellow (<u>YYYy</u>)	Yellow-green (<u>YYyy</u>)
<u>msl</u> X Lincoln	1	/	1
<u>msl</u> X Clark	13	1	5
<u>msl</u> X Dunn	6	/	0
<u>msl</u> X Dunfield	3	/	0
Total	23	1	6

Y=Y11, y=y11

* This group may contain both green and green-yellow progeny.

Green-yellow plants could be differentiated from green plants in the F2 population by segregation for plant color. Only 4 F1 plants among this group have been identified at present according to whether or not plant color segregates in F2 population. Three crosses with Lincoln, Clark, and Dunn, respectively, gave all green F2 plants and one cross with Clark gave green F2 plants and

yellow-green F2 plants indicating that the original parent was green-yellow (YYYy).

All the F1 plants were tetraploids and no triploids have been found. This result confirmed the previous observations that triploid progeny in soybean could not be produced by crossing diploid plants with tetraploid plants. It also shows that viable embryos cannot represent independent ploidy level from primary endosperm. The success of the embryo depends on normal development of the endosperm.

Tetraploid soybean can produce fairly normal progeny and is expected to have diploid gametophyte cells during meiotic division (Nirad and Vidyabhusan, 1960). As we know, the egg in normal diploid soybean plants is haploid due to meiotic division. In crosses of msl diploid (female) and tetraploid (male), therefore, a diploid egg cell must be formed to give rise to tetraploid progeny. We wanted to determine the origin of the diploid eggs in these msl msl plants. If the egg cell was directly produced from a megaspore mother cell (MMC), the F1 plants should be all green-yellow (Yllyllyllyl), because green tetraploid parent produced pollen with Yllyl and MMC in diploid msl msl plants was Yllyl. No green (Yllyllyllyl) or yellow-green (Yllyllyllyl) plants should be found.

Our results showed that all three possible phenotypes of plant color appeared in the F1 population. This indicated that the MMC's underwent meiotic division but not normally, which led to mature megagametophytes with more than the normal number of nuclei (8). Each nucleus contained either Yl or yl. The fusion between any two of those nuclei could produce a diploid egg with Yllyl, Yllyl, or yllyl genotype which would give rise to green (Yllyllyllyl), green-yellow (Yllyllyllyl), or yellow-green (Yllyllyllyl) plants in F1 population.

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10) Insertion of the transposable element Ac into soybean by transformation.

Several insertion elements (the Tgm family; Vodkin et al., 1983; Rhodes and Vodkin, 1985) and a transposable element (Groose et al., 1988) have been identified in the soybean. However, the Tgm family does not transpose and the element identified by Groose et al. has not been characterized at the molecular level. We have thus inserted the well characterized transposable element Ac from maize into soybean for eventual gene tagging studies.

Materials and methods: We obtained transformed calli by using the Agrobacterium technique of Hinchee et al. (1988). A histochemical assay was used to detect transformation, using the GUS gene. The GUS gene was placed adjacent to a selectable marker gene, NPTII within the T-DNA region of a binary vector pZA3 to generate pZAc1 (Fig. 1). pZAc1 has the 5.1 kb Ac fragment inserted in the untranslated leader region of the GUS gene, thus preventing gene expression except after the Ac element has excised. The GUS gene is driven by the CaMV 35S promotor. DNA was extracted from the soybean calli after growth for three weeks on selective media, digested with EcoRI and PstI and transferred to nylon membranes for hybridization to the entire fragment of the Ac element. Controls included DNA from the vector containing the Ac element, untransformed callus tissue and tissue transformed with a vector containing a Ds element in the untranslated leader region of the GUS gene.

Results and discussion: The binary vectors pZAc1 and pZAc3 were mobilized into A. tumefaciens strain A281 and soybean cotyledons were infected. After 10 days of growth on B5BA media containing 250 ml/L of kanamycin, the sterile actively growing calli tissue was removed and stained by x-glcU. Blue spots were scored through the dissecting microscope. The calli transformed with intact GUS gene showed even staining of the tissue. Not all, but a majority of the cells were transformed. In contrast, calli transformed with the Ac element in the untranslated leader region of the GUS gene (pZAc1) showed staining only rarely, while calli transformed with a vector containing a Ds element in the untranslated leader region of the GUS gene showed no staining. These data suggest that the Ac element is active in soybean tissue, but does not prove that Ac has integrated into the genome.

Additional proof that the Ac element had transposed in soybean tissue was obtained by hybridization. DNA extracted from the tissue was hybridized with a radioactive Ac DNA. Soybean DNA was digested with AcoRI and PstI and after hybridization with Ac DNA yielded two fragments of 4.4 kb and 3.7 kb, if the Ac DNA had not transposed. Different sized fragments from the original insertion event indicate a transposition event. Hybridization to four different preparations of DNA from different calli yielded additional bands at 5.1 kb, 2.8 kb, 2.4 kb, 1.9 kb, and 1.7 kb. These bands were much fainter in intensity due to the low concentration of these sequences. Fainter bands would be expected from

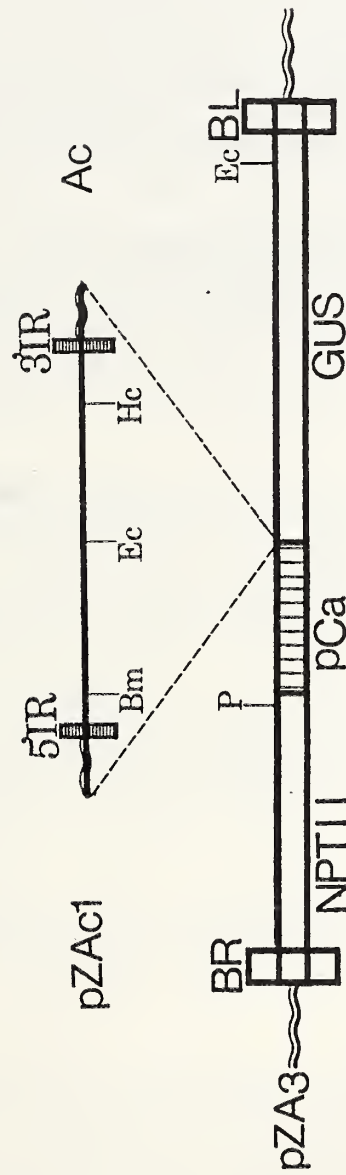


Fig. 1. Construction of plasmids: pZAc1, pZA3.
 pCa: Camv 35's promoter; IR: inverted repeat sequence;
 BR, BL: T-DNA border. Bm: BamHI; Ec: EcoRI.

transposition events since the event is less frequent than an insertion. These data strongly suggest that the Ac transposable element is active in soybean calli. We have begun regeneration of transformed tissue into whole plants with the goal of using this system for Ac tagging of useful genes.

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 1) Soybean cultivar selection on the basis of NADH oxidation: selection scheme, enzyme characteristics, and H⁺ efflux.

Selection of soybean [*Glycine max* (L.) Merr.] cultivars for improved agronomic performance can be facilitated through a basic understanding of physiological processes. This study focuses on NADH oxidation activity and H⁺ efflux in roots of soybean cultivars in relation to potential performance in the field.

Increased soybean yield and high nutrient absorption (Leggett and Frere, 1971) probably result from physiological adjustments that enhance nutrient ion uptake. Studies with barley (*Hordeum vulgare* L.) show that increased ion uptake parallels an exchange of hydrogen ions from roots for cations in the surrounding solution (Hoagland and Broyer, 1940). Soybean nutrient ion uptake may also parallel ion exchange, but the source of H⁺ has not been completely revealed.

Ion exchange is linked to NADH oxidation in corn (*Zea mays* L.) root protoplasts (Lin, 1982). In soybeans, consumption of H⁺ by total root NADH oxidation ($\text{NADH} + \text{H}^+ \rightarrow \text{NAD}^+$) may be paralleled to the lack of ion exchange in low-yielding cultivars. Previously reported data from this work (Bidlack and Stutte, 1988) indicate that low dry matter yield of a soybean cultivar may be a reflection of high NADH oxidation.

This study uses NADH oxidation as a selection criterion for finding soybean cultivars that demonstrate a negative relationship between root enzyme activity and shoot dry matter yield. The NADH oxidizing system is partially characterized in this report and the roots of selected cultivars are subjected to H⁺ efflux experiments.

Materials and methods: Three experiments were conducted: 1) preliminary screening for NADH oxidation and dry matter yield, 2) selected-cultivar experiment for enzyme characterization, and 3) H⁺ efflux on selected cultivars.

Bulk seeds of ten cultivars were obtained from the Delta Branch Mississippi Experiment Station for all experiments conducted at the Altheimer Lab in Fayetteville, Arkansas. Germination took place in an environmental chamber and the seedlings were transferred to an aerated nutrient solution (Hoagland and Arnon, 1950) at the VC to V1 stage (Fehr and Caviness, 1977) for continued growth in the chamber. Environmental conditions were as described (Bidlack and Stutte, 1988).

Placement of the plants in the chamber for the preliminary experiment followed a blind factorial lattice design with three replications. Plants for the selected-cultivar and H^+ efflux experiments were placed in a randomized complete block design with three replications. Data were evaluated at the $P = 0.05$ level.

Enzyme assays were conducted as previously described (Bidlack and Stutte, 1988) for all studies. Evaluation of NADH oxidation and dry matter yield from the preliminary screening experiment enabled selection of cultivars for further study.

Special enzyme assays in the selected-cultivar experiment included heat and sulphydryl inhibitor treatment before addition of NADH. Root tissue for the assays was obtained from 0.30 g of roots within 2.0 cm of the root tip and randomly divided into three 0.10-g representative samples for crude extraction. While the control tissue solution remained untreated, one solution was mixed with 5.0 mg of DIAMIDE [diazene-carboxylic acid bis(N,N-dimethylamide)] before centrifugation and the other treatment was boiled on a covered water bath for 5 min after centrifugation. After reaching room temperature, the three enzyme preparations were assayed for NADH oxidation activity.

Selected-cultivar plants for H^+ efflux studies were analyzed at the V1 to V2 stage. After being grown in nutrient solution, the plants were transferred to a solution of 0.50 mM $CaSO_4$ and 0.10 mM K_2SO_4 . The pH of this solution was adjusted to a pH of 7.00 ± 0.25 before the plant was immersed, but the pH varied as much as 0.25 to 3 units only minutes after transplanting. Plants remained in solution for 24 h in the environmental chamber while pH was monitored (Ramirez, 1985).

Results and discussion: Preliminary screening of ten soybean cultivars gave NADH oxidation rates and dry matter yields shown in Table 1.

Table 1. Cultivar ranking scheme for NADH oxidation in soybean roots at the V2 to V3 stage.

Cultivar (NADH group)	Root NADH oxidation (μ moles/mg/min)	Shoot dry matter yield (mg)
Davis (low)	7.8 ¹	700
Forrest (low)	8.9	630
PI88788 (low)	9.1	350
Narrow (medium)	9.3	480
Tracy-M (medium)	9.3	470
Mack (medium)	9.9	460
Dare (medium)	10.2	550
Jeff (high)	10.9	550
Pershing (high)	11.0	380
Epps (high)	14.9	650

¹No significant differences encountered in this experiment.

An inconsistent relationship between NADH oxidation and yield necessitated selection of cultivars that demonstrated the anticipated negative correlation. Elimination of some cultivars was justified on the basis of erratic results and highly variable enzyme activities. From the remaining cultivars, 'Forrest', 'Mack', and 'Pershing' were selected to represent low, medium, and high NADH oxidation rates.

In the selected-cultivar experiment, significant differences in NADH oxidation rates were found among Forrest, Mack, and Pershing (Table 2). The order of NADH oxidation activity follows that encountered in the screening experiment. It is apparent from these results that significant differences in NADH oxidation activity for the cultivars selected are obtainable only after preliminary selection of the desired cultivars from the cultivars with high variability.

NADH oxidation rates for the three cultivars were retarded by boiling the tissue solution and reversed (negative values) by sulfhydryl inhibitor (DIAMIDE) treatment (Table 2). This indicates that a heat-sensitive, sulfhydryl (SH)-dependent enzyme was responsible for oxidizing NADH in the soybean roots studies. The apparent SH dependency in these roots agrees with results by Lin (1984) of an SH-dependent NADH oxidase in corn roots. Implications of SH-dependent NADH oxidation in soybean roots may be linked to the association of SHs to increased metabolic rates in plants (Jocelyn, 1972). More work on specifically located NADH oxidases will be needed before the SH-NADH relationship in soybeans can be completely understood.

Table 2. Shoot dry matter yield, root NADH oxidation, and response of root NADH oxidation to heat and DIAMIDE treatment in three soybean cultivars.

Cultivar	-- (From Table 1) --		----- NADH oxidation -----		
	Dry wt. (mg)	NADH oxidation (μ moles/mg/min)	(μ moles/mg/min)		
			Control	Heat	DIAMIDE
Forrest	630	8.9	6.7a ¹	0.32a	-3.61b
Mack	460	9.9	12.3b	2.76b	-3.75b
Pershing	380	11.0	15.3c	0.73a	-2.69a

¹Means within a column followed by the same letter are not significantly different in this experiment.

A representative plot of H⁺ efflux is depicted in Figure 1. This plot shows that the initial H⁺ efflux (indicated by pH drop) in all cultivars remains fairly constant during the day. However, H⁺ efflux in Mack and Pershing increases just before light is removed. Compared with Forrest, an immediate H⁺ efflux is more noticeable in Pershing and Mack as is their apparent H⁺ influx later on during the night. Forrest soybean roots appear to continue H⁺ efflux even after the lights are turned on again. Perhaps

continued H^+ efflux by Forrest indicates that it has better nutrient efficiency and better potential yield compared to Pershing and Mack.

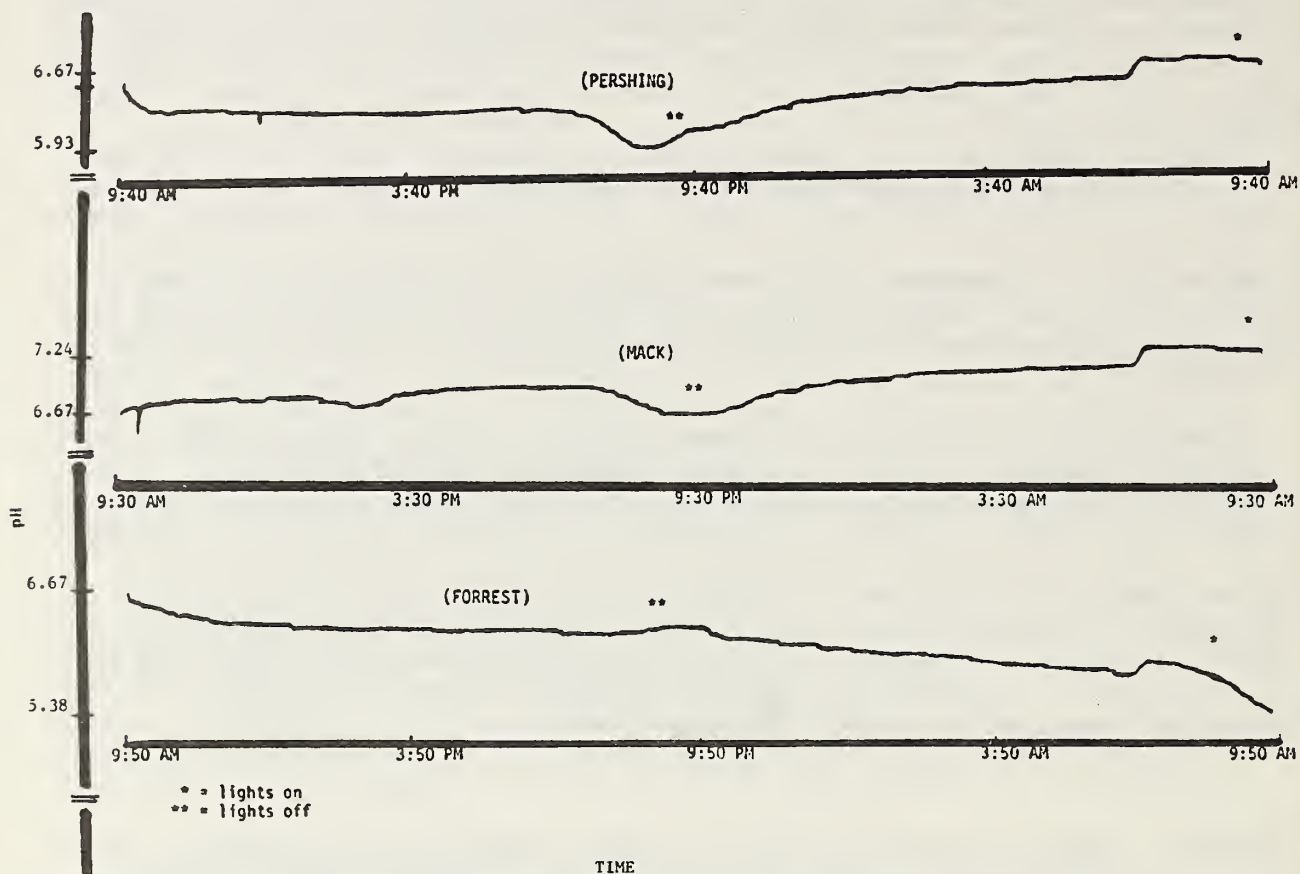


Figure 1. H^+ efflux plots of three soybean cultivars at the V1 to V2 stage.

The relation between H^+ efflux and potential nutrient uptake is supported by investigations that indicate positive correlations between H^+ efflux and K^+ influx in corn (De Quintero, et al., 1984) and in barley (Glass, et al., 1980) roots. Positive correlations have also been shown between H^+ efflux and seed yield in six soybean cultivars (Ramirez, 1985). These investigations do not show precisely when H^+ efflux occurs. Results from this three-cultivar experiment give evidence that H^+ efflux efficiency is different in Forrest because this cultivar effluxes H^+ at a slow continuous level at night.

Soybean roots may have a lower tendency to oxidize NADH when there is a principal effort to increase dry matter accumulation. Kishitani and Shibbles (1986) show that a high-yielding soybean cultivar distributes less dry matter to the roots and also has a lower total root respiration than a low-yielding cultivar. Perhaps NADH oxidation influences potential soybean yield through regulated nutrient ion exchange.

A hypothetical mechanism by which NADH oxidation regulates some active ion transport is summarized in Figure 2. In essence, NADH oxidation reduces the potential for increased yield by pulling H^+ away from the pool for ion

exchange and by diminishing the supply of reducing power of NADH used to drive metabolism. External factors such as stress, disease, and other oxidative processes decrease yield because they increase NADH oxidation, thereby reducing the level of NADH that can be stored for future metabolic demands. In Figure 2, the strong man represents NADH and the treadmill demonstrates the battle against oxidative forces that deplete the H^+ and NADH supply.

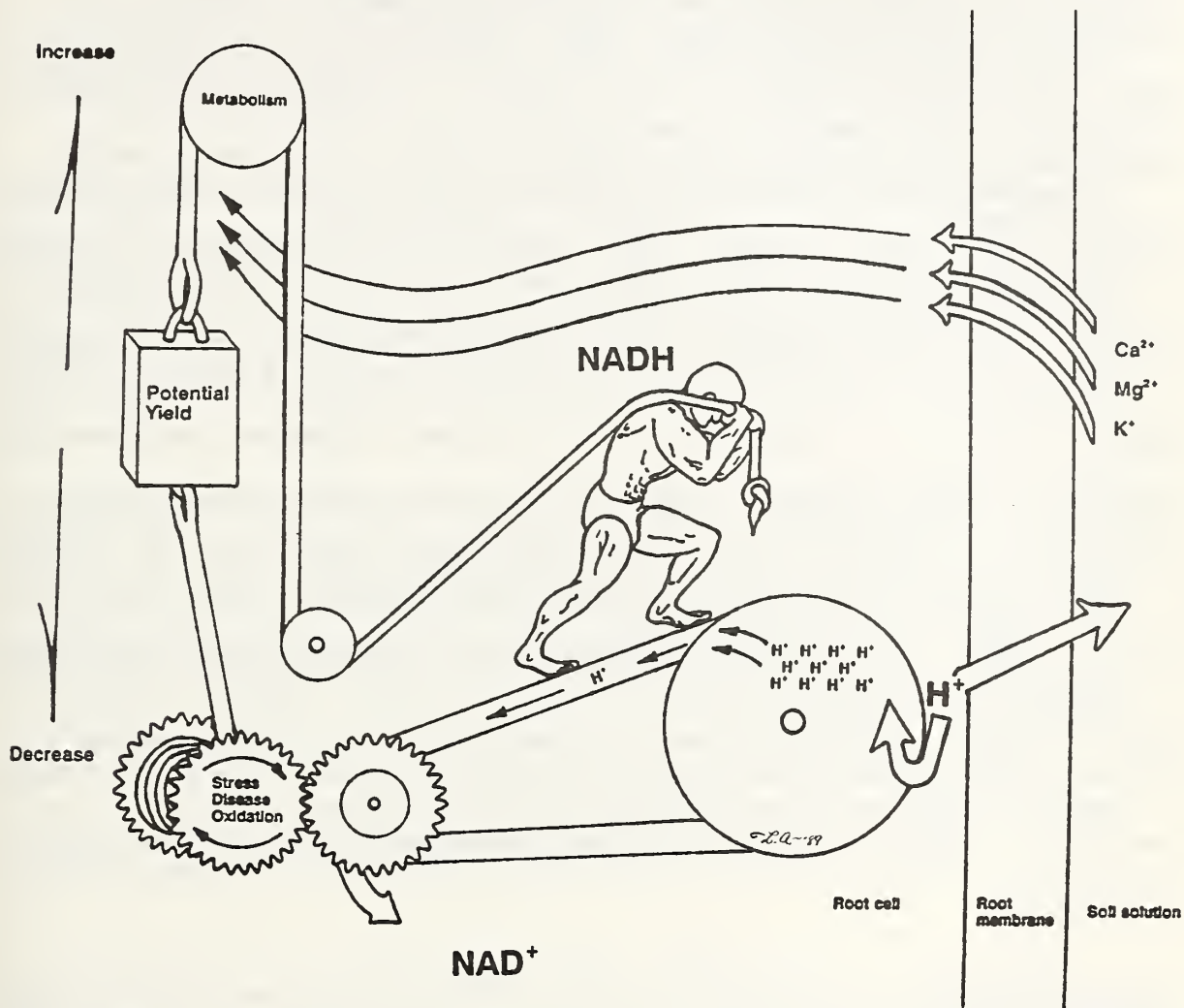


Figure 2. Hypothetical mechanism for NADH oxidation-mediated ion transport in soybean roots and its relation to metabolism.

Conclusions: Forrest, Mack, and Pershing soybeans have been selected from ten cultivars under the defined conditions to give a negative relationship between dry matter yield and NADH oxidation. These selected cultivars have significant differences in NADH oxidation activity, which can be attributed to enzyme(s) which are heat-sensitive and SH dependent. The possible mechanism for differences in NADH oxidation ability, H^+ efflux characteristics, and metabolic efficiency is outlined in a hypothetical mechanism.

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1) Harvest index, yield and physiological characteristics of soybean as related to seed size.

Harvest index (HI) influences economic yield more than any other yield determining plant trait. Donald and Hamblin (1976) have reviewed the history of the concept of harvest index and also the relationship of biological yield and HI and concluded that HI may be used as a criterion for the evaluation of cereal crops. Significant correlations have been observed between HI and yield of field crops (Schapaugh and Wilcox, 1980; Singh and Stoskopf, 1971). The objective of this study was to evaluate soybean germplasm differing in seed size for harvest index, yield, leaf area index, and phytomass.

Materials and methods: Three soybean field experiments were planted May 19, 1988, at the Agricultural Research Station, Fort Valley State College. In experiment I, 15 genotypes were planted in 3-row experimental plots in a randomized complete block design, with four replications. These genotypes consisted of 5 large seeded (18 g/100 seed or more), 5 small seeded (10 g/100 seed or less), and 5 medium seeded (in between the two grades) genotypes. Augmented design (Federer and Raghavarao, 1975) was used for experiments II and III, having 33 small seeded and 21 large seeded genotypes, respectively, in addition to 5 check genotypes that were common to both experiments. For both experiments, single-row plots were used except the 5 check genotypes ('Gordon', 'Rocky', SC82-1672, TN4-86, and 'Vance') which had 3-row plots.

The rows were 3 m long and 0.9 m wide. Twenty kg N ha⁻¹ was applied before planting. Four irrigations, 2.5 cm each, were applied during the dry periods. Plant samples were taken Aug. 26 and 30 for experiment I and Aug. 31 for experiment III. At maturity, 1-m long strip was harvested (from Oct. 17 to Nov. 7) from the middle row in experiment I. In experiments II and III, 1-m strip was harvested from each experimental row and the middle row of check genotypes. The plant material was dried to a constant weight in a drier at 40 C before hand threshing the seed. Harvest index (%) was determined by dividing the seed by seed-plus-stems-and-branches. Phytomass (MT ha⁻¹) was calculated from total dried above-ground material obtained from 1 m of row length, approximately 104 days after planting. All the leaves and most of the petioles had fallen at harvest. All data were subjected to analysis of variance.

Results and discussion: Seed yield, HI, leaf area index (LAI), and phytomass differed significantly among the genotypes in experiment I (Table 1). TN5-85 had significantly higher yield than all the other genotypes except SC82-1672. Phytomass ranged from 7.13 to 16.49 MT ha⁻¹.

Table 1. Seed yield, harvest index, leaf area index and phytomass of soybeans in experiment I during 1988.

Genotype	Seed size	Yield MT ha ⁻¹	Harvest index (%)	Leaf area index (ratio)	Phytomass MT ha ⁻¹
TN5-85	medium	4.48	43.08	3.48	7.61
SC82-1672	small	4.07	36.08	9.77	12.87
Rocky	small	3.04	32.84	7.04	12.02
Gordon	small	3.01	29.14	10.60	15.93
D71-V89	large	2.97	33.32	7.18	8.68
D71-V86	large	2.97	40.09	8.31	10.11
G81-152	medium	2.84	26.69	9.27	11.20
TN4-86	small	2.82	41.08	3.31	8.97
Late-Giant	large	2.66	25.64	8.88	10.82
Tokyo	large	2.60	37.02	10.04	11.41
G80-1413	medium	2.51	27.45	9.64	14.22
Epps	medium	2.48	27.89	7.42	12.36
Vance	small	2.38	38.16	4.46	7.13
Tanbagura	large	2.05	20.23	8.90	11.83
G81-234	medium	2.01	22.78	11.15	16.49
Mean		2.86	32.10	7.96	11.44
LSD (0.05)		1.22	12.92	3.72	4.78

Yield ranged from 2.01 to 4.48 MT ha⁻¹ for G81-234 and TN5-85, respectively. The HI for TN5-85 was significantly higher than many other genotypes. The LAI for TN5-85 and TN4-86 was lower than all other genotypes and these two genotypes had the highest harvest index. Apparently, TN5-85 partitioned more photosynthate to seed as compared with other genotypes. Most of the low yielding genotypes appear to produce more foliage, which may decrease photosynthate by mutual shading. The effects of seed size on harvest index, yield, leaf area index, and phytomass were not significant even though there was a trend for harvest index and yield to decrease, and LAI and phytomass to increase with increasing seed size.

In experiment II (small seeded genotypes), HI and yield ranged from 13.63 to 43.68, and 2.13 to 6.61 MT ha⁻¹, respectively, and the corresponding values in experiment III (large seeded genotypes) were 23.91 to 56.59 and 1.40 to 6.00 MT ha⁻¹, respectively (Table 2). Leaf area index and phytomass also differed significantly among the genotypes. The mean yield and HI for the three experiments are similar. This indicated that the augmented design may be used for soybean germplasm evaluation when seed is limited.

Harvest index and yield exhibited significant positive correlation ($r=+0.62$) in experiment I but not in experiment III. In experiment II, a positive correlation ($r=+0.31$) was observed between harvest index and yield which was significant at the 8% level of probability. LAI and phytomass were significantly correlated in experiments I and III. Leaf area index was not determined in experiment II. In experiment I, a significant negative correlation ($r=-0.46$) was observed between harvest index and LAI. The significant correlations between HI and yield indicated that harvest index might be useful in evaluation of soybean germplasm for identification of superior and higher yielding genotypes.

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Table 2. Yield and harvest index of small and large seeded soybeans during 1988.

Experiment II			Experiment III		
----Small seeded genotypes-----			----Large seeded genotypes-----		
Genotype	Yield	Harvest index	Genotype	Yield	Harvest index
	MT ha ⁻¹	(%)		MT ha ⁻¹	(%)
Pershing	6.61	43.67	Tanbagura	6.00	33.95
PI 196177	6.20	41.49	D71-V89	5.28	31.95
PI 398583	6.05	42.98	PI 417128	3.62	34.85
PI 171437	5.68	31.95	PI 417213	3.38	56.59
PI 181697	5.57	33.60	Tokyo	3.36	30.38
PI 398269	5.46	39.49	PI 181565	3.18	33.02
PI 393550	5.25	32.83	H86-6865	3.04	26.33
PI 393547	4.94	27.64	D71-V86	2.88	25.77
PI 416867	3.87	32.93	Rockusun	2.59	30.34
PI 324068	3.61	30.39	H86-8097	2.48	23.91
PI 194773	3.51	29.32	PI 230972	2.48	30.92
PI 416900	3.40	26.10	PI 417359	2.36	35.14
H86-5824	3.25	35.90	PI 416893	2.34	28.96
PI 408155	3.19	39.65	PI 416982	2.15	32.03
PI 222397	3.10	24.13	PI 417288	2.14	38.08
H86-5984	3.10	36.29	D74-9820	2.07	27.30
PI 427241	2.97	23.35	PI 423758	1.91	33.33
H86-5709	2.90	39.10	PI 417440	1.80	31.71
H86-5584	2.83	29.83	Hahto	1.68	30.14
Laredo	2.82	31.28	PI 417159	1.61	28.23
PI 423852	2.78	36.95	PI 417310	1.40	31.26
PI 398479	2.63	38.96			
D59-2537	2.50	36.69	MEAN	2.75	32.10
			LSD (0.05)	2.95	15.98
PI 423827B	2.49	34.74			
PI 417193	2.48	39.74			
N83-640	2.36	28.14			
PI 416771	2.35	38.26			
PI 227687	2.34	13.63			
PI 86490	2.33	33.62			
PI 417052	2.31	40.18			
PI 494181	2.27	28.52			
PI 423759	2.25	32.37			
H86-5708	2.13	34.42			
MEAN	3.50	33.58			
LSD (0.05)	0.99	13.20			

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1) Test for linkage between fan and w loci.

The linolenic acid concentration of soybean genotype Cl640 is reduced approximately 50%, compared with typical soybean cultivars due to the homozygous recessive condition at the fan locus (Wilcox and Cavins, 1987). Genotypes heterozygous at this locus (Fan fan) have linolenic acid concentrations intermediate between the two homozygous types (Wilcox and Cavins, 1985). While initiating the backcrossing of allele fan from Cl640 into 'Pennyrile', nine F₂ seeds with less than 4% linolenic acid (fan fan) were planted to use for the first backcross. All nine plants had purple flowers (W _). Because Pennyrile has white flowers (w w) and Cl640 has purple flowers (W W) this suggested that the fan locus may be linked to the w locus.

We subsequently recorded seed linolenic acid concentration and flower color on 566 F₂ plants from the repulsion phase cross Pennyrile x Cl640. The phenotypes at the w locus segregated 3:1 ($X^2 = 0.68$, $0.50 > P > 0.25$), and the phenotypes at the fan locus segregated 1:2:1 ($X^2 = 0.80$, $0.75 > P > 0.50$). The chi square value (Table 1) for the dihybrid cross indicates the fan and w loci assort independently and are unlinked.

Table 1. F₂ data from soybean dihybrids segregating for the fan and w loci.

Genotype						n	Probability X ² level	
<u>Fan Fan</u>	<u>Fan fan</u>	<u>fan fan</u>	<u>Fan Fan</u>	<u>Fan fan</u>	<u>fan fan</u>			
110	212	117	34	84	32	566	6.4	0.50 > P > 0.25

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 2) Recombination rate and the selfing environment//

Most attempts to address the question of the value of increasing genetic recombination in plant breeding have utilized an increased number of generations of intermating to increase the genetic recombination (Piper and Fehr, 1987). Recombination rates, however, can also be affected by both the genotype undergoing meiosis and the environment during meiosis (Stadler, 1926; Allard, 1963). This report compares recombination rates between the linked alleles ln and p2 following selfing in several natural environments.

The soybean variety development program at the University of Kentucky could be set up to self F1 plants in either the summer or winter months. Therefore, three or four years of selfing environments were compared in the following locations: Lexington, KY field environments with flowering in July; Lexington greenhouse environments with flowering in February; and Isabela, Puerto Rico (Iowa State University - University of Puerto Rico winter nursery) field environments with flowering in December. At the field environment in Lexington, different planting dates could be used that would alter the time of flowering but still allow the production of an adequate number of seeds per plant. The planting dates 22 May, 5 June, and 19 June were compared in two years at the Lexington field environment for the influence on recombination rates.

The genes ln, narrow leaf, and p2, puberulent plant, are assigned to linkage group 4 with the reported recombination rate of 26.4±1.4% (Bernard and Weiss, 1973). The coupling linkage ln p2 was selected from the cross of 'Clark' isolines L70-4049 (p2) and L62-1579 (ln) supplied by Dr. R. L. Bernard, University of Illinois. The determination of recombination rates for selfing in each environment was based on screening F2 plants from the cross Clark ln p2 x Clark and calculated by the method of Immer and Henderson (1943). There were three exceptions to this: the Lexington greenhouse 1983 data were from the cross in repulsion, and the data for the Lexington greenhouse 1988 and Puerto Rico field 1987 were obtained by screening F3 progeny of F2 plants (genotype Ln P2 ln p2) from the cross Clark ln p2 x Clark.

Recombination rates for individual environments ranged from 24.1 (n=1099) for selfing in the Lexington field 1985 environment to 38.9 (n=797) for selfing in the Lexington greenhouse 1988 environment (Table 1). Although significant differences existed between recombination rates in individual environments, an analysis of variance indicated no significant differences among the three locations for mean recombination rate. Likewise, while the recombination rate calculated for the 5 June 1987 planting date was significantly greater than that of the other two planting dates from that year and from the recombination rate for the 5 June 1986 planting date, an analysis of variance indicated no significant differences among the three planting dates for mean recombination rate (Table 2).

Table 1. Recombination rates at the ln p2 linkage group with selfing in different locations.

Selfing location	Year	Recombination rate	No.
Lexington-Field	1984	26.9 \pm 2.5	420
Lexington-Field	1985	24.1 \pm 1.5	1099
Lexington-Field	1986	31.3 \pm 1.8	1007
Lexington-Field	1987	29.7 \pm 1.6	1095
Lexington-Field	\bar{x}	28.0	
Lexington-Greenhouse	1983	28.0 \pm 4.3	428
Lexington-Greenhouse	1986	25.5 \pm 1.9	707
Lexington-Greenhouse	1988	38.9 \pm 2.2	797
Lexington-Greenhouse	\bar{x}	30.8	
Puerto Rico-Field	1984	26.9 \pm 1.6	933
Puerto Rico-Field	1985	27.5 \pm 1.5	1127
Puerto Rico-Field	1986	28.3 \pm 4.6	134
Puerto Rico-Field	1987	37.7 \pm 1.6	1378
Puerto Rico-Field	\bar{x}	30.1	

Table 2. Recombination rates at the ln p2 linkage group from selfing in Lexington field environments that differed due to differing planting dates

Planting date	Year	Recombination rate	N.
22 May	1986	30.1 \pm 2.7	431
22 May	1987	29.7 \pm 1.6	1095
22 May	\bar{x}	29.9	
5 June	1986	28.8 \pm 4.4	146
5 June	1987	38.4 \pm 2.1	902
5 June	\bar{x}	33.6	
19 June	1986	33.6 \pm 2.8	430
19 June	1987	29.3 \pm 1.2	1884
19 June	\bar{x}	31.4	

The environment has the potential to cause shifts in recombination frequency. That was seen here with the ln p2 linkage group and the diverse environments investigated. While it is tenuous to extrapolate from one linkage group to the entire genome, there was no indication that these macroenvironmental differences could produce predictable increases or decreases in recombination rates that could be utilized in the soybean breeding program. These results are similar to those reported by Allard (1963) for lima bean, where differences were observed for recombination frequency among three different planting dates but a consistent pattern in the fluctuation of recombination frequencies between different dates was not revealed.

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 1) Evaluation of certain specialty soybean germplasm for corn earworm resistance and harvest index. //

The expanding market of the soyfood industry is generating considerable interest for the development of specialty soybean varieties. One major factor that hinders profitability is damage by insect pests. Corn earworm (Heliothis zea Boddie) is a major insect pest that damages soybeans and reduces its yield and market value. Heavy infestation of corn earworm can cause complete crop loss if control measures are not applied. This pest feeds on both the foliage and developing seeds in the pod. Soybeans become primary host as corn (Zea mays) and cotton (Gossypium hirsutum) become mature and consequently less attractive for oviposition (Freeman et al., 1967). Each corn earworm larva is capable of damaging 6 to 8 pods and about 7 seeds between 4th and 6th instars (Boldt et al., 1975; Smith and Bass, 1972). Cost of chemical control, environmental hazards, and, most of all, concerns of consumers to use chemically treated food products leave scientists with no choice but to develop resistant cultivars to this and other insect pests of soybeans. One objective of this study is to identify specialty soybean germplasm that is resistant to corn earworm damage.

The proportion of biological yield represented by economic yield was defined as harvest index (HI) by Donald (1962) and as seed yield efficiency (SYE) by Joshi and Smith (1976). There is evidence that improvement in yield of crops has resulted in part from unconscious selection for high HI, especially where the reproductive parts constitute economic yield. Van Dobben (1962) demonstrated that, in 50 years of wheat breeding, the grain : straw ratio increased from 0.51 to 0.66, representing a 29% increase in yield. Similar changes have been observed in barley (Thorne, 1958; Watson et al., 1958, and 1963), rice and peas (Donald, 1962), corn (Stinson and Moss, 1960; Sowell et al., 1961) and dry beans (Wallace and Munger, 1966). Manipulation of HI, which determines biological efficiency of a genotype, would enable us to develop cultivars with high economic returns. As the price of inputs increases, the economic yield becomes more crucial. The second objective of this study is to identify specialty soybean germplasm with high HI.

Materials and methods: Thirty four specialty soybean cultivars belonging to Maturity Groups III and IV were evaluated for their resistance to corn earworm, and HI in 1988. These 34 cultivars consisted of 11 large seeded entries in Maturity Group III (Table 1), 10 small seeded entries in Maturity Group IV (Table 2) and 13 large seeded entries in Maturity Group IV (Table 3). Each cultivar was planted in a single-row plot, rows being 60 cm long and 75 cm apart. Cultivars were planted July 28, 1988, in a randomized complete block design with three replications. Plants were harvested at ground level on Oct. 21, 1988. Four plants were randomly selected from each row for observations on pod damage and HI. After pod damage count, all the samples were dried at 50 C for 12 h. HI was calculated by dividing economic yield, i.e., seed weight, by total biological yield excluding the weight of leaves, petioles, and roots.

Experimental results: Data for each cultivar for Maturity Groups III and IV for Harvest Index and percent pod damage by corn earworm are given in Tables 1, 2, and 3.

The range of HI in Maturity Group III cultivars varied from 0.50 to 0.58. Cultivars 'Kim' and 'Pella' gave the highest HI and 'Kura' had the lowest HI out of the 11 cultivars (Table 1).

Cultivars in Maturity Group IV were separated into two groups according to seed size, i.e., large seeded or small seeded. Data for small seeded cultivars are given in Table 2; for large seeded cultivars in Table 3. Among the small seeded cultivars, the HI ranged from 0.44 to 0.56. The highest HI was obtained from cultivar 'Kingston' and lowest from PI 85505. Among the large seeded cultivars (Table 3) HI ranged from 0.48 to 0.56 and cultivar 'Sanga' had the highest HI. Cultivar 'Emerald' gave the lowest HI.

The range of pod damage by corn earworm varied from 0.95 to 4.47% among Maturity Group III cultivars (Table 1). The most resistant cultivar was Kim and the most susceptible cultivar was 'Columbia'. Although the differences among different cultivars for HI and pod damage were not statistically significant, it appears that cultivar Kim has the highest HI and has maximum resistance to corn earworm. Statistical significance might have been lost due to small sample size, fewer replications and large variations within the replications. Pod damage among small seeded cultivars (Table 2) in Maturity Group IV ranged from 1.08 to 5.65%. The most resistant cultivar was PI 85505 and the most susceptible was 'Sooty', with 5.65% damaged pods. Among the large seeded cultivars in Maturity Group IV (Table 3) pod damage ranged from 0.67 to 10.29%. The most resistant cultivar was Sanga and the most susceptible was 'M. Summer', with 10.29% damaged pods. Cultivar Sanga had the highest HI as well as maximum resistance to corn earworm.

Preliminary data indicate that cultivars Kim and Sanga have high levels of resistance and possess high HI. These cultivars might be good source material for improving HI and corn earworm resistance in the new specialty soybean cultivars.

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Table 1. Harvest index and corn earworm pod damage for large seeded entries in Maturity Group III of soybean.

Cultivar	Harvest Index	Damaged pods (%)
Wolverine	0.55	1.74
Willomi	0.55	1.19
Fuji	0.55	1.32
Oakland	0.56	3.84
Guelph	0.54	1.69
Columbia	0.51	4.47
Kura	0.50	2.36
Kim	0.58	0.95
Williams 79	0.55	4.37
Pella	0.58	2.61
Kanrich	0.57	2.99

Tables 2 and 3. Harvest Index and corn earworm pod damage for entries in Maturity Group IV

(Table 2) Small seeded entries			(Table 3) Large seeded entries		
Cultivar	Harvest Index	Damaged pods (%)	Cultivar	Harvest Index	Damaged pods (%)
Kingston	0.56	3.31	Funk Delicious	0.50	2.05
Sooty	0.47	5.65	KaiKoo	0.52	2.92
PI 85505	0.44	1.08	Shiro	0.54	1.99
Wilson-5	0.45	2.60	M. Summer	0.52	10.29
PI 86103	0.46	4.65	Sato	0.54	2.25
PI 84751	0.48	1.87	Emerald	0.48	3.76
Peking	0.46	1.39	Emperor	0.49	1.97
Norredo	0.51	2.31	Sanga	0.58	0.67
PI 339948	0.52	1.51	Ware	0.49	4.39
PI 82264	0.55	4.30	Verde	0.45	4.41
			Kahala	0.55	4.00
			Kailu	0.55	4.31
			Aoda	0.53	3.61

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1) Salt tolerance in Glycine max and perennial Glycine //

About one third of the world's irrigated lands are affected by salt (Moore, 1984). The development of salt-tolerant crops including soybean could greatly help to meet demands on the global food supply. Soybean cultivars exhibit differential tolerance to salt during seed germination and plant growth (Abel and MacKenzie, 1964). The cultivars 'Lee' and 'Jackson' have been identified as salt tolerant and sensitive, respectively, by Abel (1969) and others (Weil and Khalil, 1986; Parker et al., 1986). The wild perennial Glycine species have been suggested as a potential source of germplasm to improve soybean for agronomic traits including salt tolerance (Brown et al., 1984). The purpose of this study was to evaluate the salt tolerance of several accessions of perennial Glycine and to compare their response with that of three soybean cultivars.

Materials and methods: Accessions from several species of perennial Glycine were screened for salt tolerance. The cultivars Lee, Jackson, and 'Morgan' were included to compare their salt reaction with the perennial accessions. The design was a split plot with salt level as the whole plot treatment and genotypes randomized within the whole plot. Salt levels of 0, 5, 10, and 15 g NaCl/L were used with three replications of each treatment.

The experiment was conducted in aquaria holding 45 L distilled water with nutrient solution modified from Johnson et al. (1957). Plants were allowed to grow in the nutrient solution for approximately three weeks at which time the three NaCl treatments were imposed. Plants were grown for approximately two weeks in the salt treatments. Visual chlorosis scores and shoot dry weight were determined on each genotype.

Results and discussion: The highest two salt treatments caused severe visual chlorosis or death for all cultivars and several of the perennial species. However, accessions of G. microphylla and G. argyrea showed only slight visual chlorosis in the 10 g NaCl/L salt treatment. Furthermore, the G. argyrea accession had only moderate visual chlorosis in the highest salt treatment.

Table 1 shows the total shoot dry weight for several genotypes grown in the 5 g NaCl/L salt treatment expressed as a percentage of the control plants. Accessions of G. tomentella, G. microphylla, and G. argyrea showed enhanced shoot dry weight at this low salt level, suggesting salt tolerance. The relative dry weights of these species were significantly greater than that of the salt-sensitive cultivar, Jackson, which achieved only 42% of its control shoot dry weight when grown in this salt treatment. The cultivar Morgan appears to have salt tolerance

equivalent to that observed in Lee at the low and at intermediate salt levels, and will be utilized in further evaluations. The G. clandestina accession appeared to be salt sensitive, attaining only 10% of the control shoot dry weight at this low salt level

This study indicates that there is considerable variation in salt tolerance among the perennial Glycine species. Further research is being conducted to characterize the reaction of the perennial Glycine species to salt stress.

Table 1. Total shoot dry weight for five plants of several genotypes grown in 5 mg NaCl/L salt treatment expressed as a percentage of each genotype's control.

Genotype	Dry wt. (Percentage of control)
<u>G. tomentella</u>	137.4
<u>G. microphylla</u>	134.0
<u>G. argyrea</u>	117.1
<u>G. max</u> cv. Morgan	106.0
<u>G. max</u> cv. Lee	85.3
<u>G. max</u> cv. Jackson	41.6
<u>G. clandestina</u>	10.0
LSD(0.10) = 60.4	

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1) Evaluation of soybean introductions for resistance to stem rot caused by *Sclerotinia sclerotiorum*, by a laboratory method.

Sclerotinia sclerotiorum (Lib.) de Bary causes stem rot or white mold, a minor but potentially destructive disease of soybean (Grau and Bissonnette, 1974). Control of this pathogen through cultural practices such as planting in widely spaced rows can reduce disease (Grau and Radke, 1984), but this practice can result in yield losses if environmental conditions are unsuitable for disease development. Since the early 1980's, stem rot has increased in importance, because of planting in narrowly spaced rows and the cropping of soybeans in fields formerly planted to other susceptible crops (Chun et al., 1987).

Researchers working with dry bean (*Phaseolus vulgaris* L.) developed a growth room method for screening plant introductions of *Phaseolus* spp., in order to identify sources of resistance that could be used for breeding bean varieties resistant to white mold caused by *S. sclerotiorum* (Hunter et al., 1981; 1982). This procedure was adapted for use in soybean by Cline and Jacobsen (1983), and was reported to be a feasible method for evaluating soybean cultivars for resistance to stem rot. However, this group apparently has not continued with screening for resistance. Boland and Hall (1986) have used the growth room assay to evaluate 43 soybean cultivars for resistance to stem rot. At Michigan State University, Chun et al. (1987) developed an assay for evaluating soybean reaction to stem rot, in which excised stems of greenhouse-grown plants were inoculated, then incubated on moist vermiculite in trays in the laboratory.

In this report, the screening of plant introductions (PI's) and wild soybeans (*Glycine soja* Sieb. & Zucc.) for resistance to stem rot by the laboratory method is described.

Materials and methods: The assay method has been described in detail (Chun et al., 1987). Briefly, soybean seeds (four per pot) were planted in 11-cm diameter X 14-cm high plastic pots of 946 cc (32 oz.) capacity containing a potting mix consisting of a steamed mixture of sandy loam soil, sphagnum peat, and sand (5:3:2, v:v:v). Plants were grown in a greenhouse for 6 wk. One week after planting, plants were thinned to three per pot. One and one-half g granular fertilizer (12-12-12, NPK) was applied to the surface of the soil approximately 1, 3, and 5 wk after planting. From late September until mid-May, day length was extended to 12 hr with high-intensity sodium lamps or fluorescent lights. The temperature in the greenhouse varied from 20 to 25 C. Wild PI's, some of which tended to be viney, were staked on bamboo rods.

Plants were cut off at the soil level, and the leaves and tops of the plants were excised, providing stem sections consisting of the bottom 25-30 cm of the plant. Excised stems were placed in plastic trays (54 X

26 X 6 cm) on moist vermiculite. Stems were inoculated by applying a disk of mycelial inoculum, grown on 2% millet seed agar, to the cut surface at the stem apices. The trays were covered with plastic film and were incubated on a laboratory bench at 21 ± 1 C for 6-9 (usually 7) days before rating. Resistance was determined by measuring the length of the lesion from the site of inoculation to its farthest extent.

A randomized complete block design was used. There were 4-11 (usually 8) replications, with one plant of each PI per replication. Data were subjected to analysis of variance, and differences between means were distinguished by Fisher's Least Significant Difference or by Tukey's Honestly Significant Difference ($P=0.05$). The cultivar 'Corsoy', which has been shown to be resistant in both growth room (Cline and Jacobsen, 1983) and field (Chun et al., 1987; Grau et al., 1982) studies, was included in all experiments.

Results: Eighty-three experiments were done, usually with 17 PI's per experiment; 886 PI's (*Glycine max*) and 109 wild PI's (*G. soja*) were screened (Tables 1 and 2). The frequency distribution of average lesion lengths of the PI's, expressed as percentages of the average lesion length of Corsoy, for comparison among experiments, is shown in Fig. 1. Six categories were arbitrarily chosen: <70%, 70-90%, 90-110%, 110-130%, 130-160%, and >160% of the average lesion length of Corsoy.

Most of the PI's had longer lesions than did Corsoy, which consistently had the shortest or one of the shortest lesions, although its average lesion length varied from 1.7 to 14.7 cm (average = 8.5) in different experiments. One hundred and forty-six cultivars were identified for further testing, either because they had short lesion lengths, or because the experiment in which they were performed had a high coefficient of variability, making retesting desirable. These have been re-evaluated two, three, and four times with varying reproducibility of results. Table 3 shows results for 94 PI's whose lesion lengths were comparable to or shorter than that of Corsoy in one or more tests. In many but not all instances, disease reaction was confirmed in subsequent testing.

The wild soybeans were tested in the hope that their greater phenotypic variability would reveal some with greater resistance than was exhibited by Corsoy and the PI's. However, none was more resistant than Corsoy by our method of testing; 49 were not significantly different from Corsoy, and 54 had significantly longer lesions. Five that were tested twice had lesions not significantly different from those of Corsoy in one test, and had significantly longer lesions in the other.

Discussion: The average lesion length of Corsoy varied from experiment to experiment, which sometimes altered the relative expression of resistance of a PI, as compared with Corsoy (e.g., see PI 54.606-2, tests 2 and 3, and PI 68.011, tests 1 and 2) (Table 3). However, in the vast majority of the experiments, Corsoy had the lowest or one of the lowest lesion lengths.

Of the 886 PI's evaluated for resistance to stem rot thus far, several have demonstrated a greater restriction of growth of S. sclerotiorum through the stem tissue than Corsoy, the resistant control. These PI's require further testing in the field. If the resistant reaction is verified, these PI's can be used as sources of resistance in a breeding program.

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Table 1. Plant introductions (PI) of Glycine max screened for resistance to stem rot by the excised stem assay.

PI Number	Maturity Group	PI Number	Maturity Group	PI Number	Maturity Group
19.986	IV	54.859	II	68.427	II
30.594	II	862	II	430	II
599	II	865	I	436	II
600	II	873	II	439	II
36.653	I	55.089-1	III	443	II
47.131	II	887	IV	446	II
54.583	III	56.563	IV	448	II
591	III	57.334	III	449	IV
592	III	58.955	IV	454	II
600	IV	59.849	IV	455	II
54.604	II	60.269-2	IV	68.457	II
606-1	IV	272	III	457-1	II
606-2	IV	279	II	461	II
607	II	296-1	II	461-1	II
608	II	296-2	III	465	II
608-1	II	970	IV	465-1	II
608-2	III	61.940	III	466	II
608-3	III	944	IV	470	III
608-4	IV	947	IV	474	II
608-5	III	62.199	IV	474-2	I
54.609	III	62.202	III	68.475	II
610-1	III	202-2	IV	475-1	II
610-4	IV	248	IV	479	III
613	III	483	III	479-1	III
614	IV	63.271	I	480	II
615	III	468	IV	481	II
615-1	III	945	IV	483	III
615-2	IV	64.698	IV	484-1	II
617	IV	747	IV	484-4	II
618	III	65.338	II	488	II
54.619	II	65.341	II	68.494	III
620	III	346	II	500	II
620-2	III	354	II	503	II
809	I	379	III	508	II
818	I	388	II	516	II
834	I	68.011	IV	521	II
853	I	398	III	521-1	III
854	I	410	II	522	II

Table 1. (Cont'd.)

PI Number	Maturity Group	PI Number	Maturity Group	PI Number	Maturity Group
855	00	421	II	523	III
857	I	423	III	526	II
68.528	III	68.670-1	II	68.761-3	III
530	II	670-2	II	762	II
530-2	III	671	II	763	II
533-1	III	676	II	765	II
533-2	III	679	III	769	IV
535	III	679-2	IV	770	I
543	II	680	II	778	II
551-2	II	680-2	II	788	II
551-3	I	683	II	795	II
554	I	685	II	806	III
68.555	II	68.687	II	68.815	II
560	III	692	IV	69.500	II
562	II	692-2	III	501	II
564	II	694	II	503	II
572	I	696	II	507	I
576	I	701	III	506-1	IV
585	II	704	II	512	II
586	I	706	II	515	III
587	II	708	II	532	II
598	II	709	II	533	I
69.599	III	68.710	III	69.991	II
600	II	712	II	992	II
604-1	III	713	II	993	III
604-2	I	715	II	995	III
609A	III	718	II	996	II
609B	II	722	0	70.001	III
610	I	725	II	009	II
621	III	728	II	013	IV
622	II	729	II	014	III
627	II	731	III	016	I
68.629	II	68.732	II	70.017	I
639	II	732-1	III	019	III
642	II	736	II	021	II
644	IV	741	II	70.023	III
648	III	746	I	70.027	I
655	II	748	II	036	II
658	II	748-1	III	076	III

Table 1. (Cont'd.)

PI Number	Maturity Group	PI Number	Maturity Group	PI Number	Maturity Group
661	II	756	III	077	II
663	II	759	III	078	II
666	II	761	II	080	III
70.084	II	70.471	III	78.243	I
087	I	473	III	79.583	III
089	II	473-1	I	586	II
70.091	II	476	II	587	III
188	III	478	II	593	II
189	III	485	I	596	II
192	III	490	IV	602	II
197	II	495	II	609	II
199	III	500	III	610	I
201	III	501	III	613	II
70.202	III	70.503	II	79.616	III
208	IV	507	II	617	I
212	III	515	III	620	III
70.213	III	516	II	627	III
224	II	519	III	628	III
228	II	520	I	645	III
229	IV	528	III	648	I
241	I	541	III	691	III
242	II	559	III	691-4	III
242-2	IV	561	II	692	III
70.242-4	O	70.566	III	79.693	III
243	IV	71.161	I	694	I
247	III	444	IV	695	II
70.251	II	463	IV	696	IV
253	III	506	IV	699	I
453	II	845	III	703	II
456	II	850	II	710	III
457	II	850-1	III	712	II
458	II	72.227	IV	726	III
459	II	232	III	727	I
70.460	II	72.328	II	79.732-3	IV
461	II	337	II	732-4	IV
462	III	341	II	737	II
463	II	342	II	739	O
466-3	IV	73.583	III	743	IV
466-4	III	585	II	745	II
467	IV	587	II	746	II

Table 1. (Cont'd.)

PI Number	Maturity Group	PI Number	Maturity Group	PI Number	Maturity Group
469	III	772	II	747	II
469-1	III	780	II	756	II
470	III	78.242	II	760	III
79.761	II	80.828-2	IV	81.764	IV
773	II	831	III	765	I
797	III	834-1	IV	766	III
825-1	IV	834-2	IV	767	II
835	III	837	IV	768	II
846	II	841	III	770	II
848	II	844-2	III	771	II
848-1	III	844-3	III	772	I
862-1	II	845-1	III	773	II
863	II	845-2	III	775	I
79.870-1	I	80.847-1	III	81.777	IV
870-2	III	847-2	IV	780	III
870-4	IV	81.023	IV	785	III
870-6	IV	027	III	971	II
872	III	029-1	IV	82.183	I
874	III	029N	II	184	II
874-1	III	030	IV	210	IV
885	II	030-1	III	218	IV
80.459	III	031-1	III	232	III
461	III	031-2	III	235	III
80.466-1	III	81.033	I	82.246	IV
466-2	IV	034-1	IV	246-1	III
469	II	034-2	IV	259	IV
470	III	035	II	263-1	IV
471	II	037-1	IV	263-2	II
471-1	III	037-2	III	263-3	II
473	IV	037-3	III	264	IV
479	IV	037-4	I	278	III
480	III	037-5	IV	291	IV
481	III	038	III	295	IV
80.485	II	81.040	I	82.296	IV
488	IV	041	III	302	III
488-1	II	041-1	III	307	IV
494	II	042-1	IV	308	III
498-1	IV	042-2	IV	312N	IV
536	II	044-1	III	315	IV
671	II	044-2	III	325	IV

Table 1. (Cont'd.)

PI Number	Maturity Group	PI Number	Maturity Group	PI Number	Maturity Group
822	III	667	III	326	IV
825	III	761	III	509	IV
828-1	IV	763	II	527	IV
82.532	II	84.964	I	87.524	II
534	IV	965	II	531	I
544	IV	992	II	619-1	II
554	IV	85.012	II	628	II
555	IV	014	II	631	II
558	IV	340	II	88.288	I
581	IV	492	II	293	II
83.853	IV	508	II	293A	II
858	IV	625	II	294	II
868	IV	671	II		
				88.294-1	II
83.881	IV	86.002	II	295	I
881A	IV	021	I	295-1	II
889	IV	022	II	296	II
891	IV	023	II	298	II
892	IV	031	II	301	II
893	IV	038	II	303	II
915	IV	045	II	304	II
923	IV	046	II	307	II
925	IV	050	II	308	II
945-3	I				
		86.069	II	88.309	II
84.580	II	089	II	311	II
609	II	102	II	313	II
637	II	112	II	351	II
665	II	113	II	352	II
666-1	II	115	II	355	II
668	I	122	II	356	II
668-1	II	133	I	357	II
673	II	137-1	II	358	II
673-1	II	410	I	442	II
674	I				
		86.411	I	88.443	I
84.681	II	416	I	455	II
683	II	443	II	468	II
683A	II	454	II	479	II
686	I	463	II	484	I
750	II	737	I	495	II
810	I	741	II	497	I
896	II	878	II	508	II
921	II	878-2	II	777	II
928	II	972-1	II	787	II
954	II	87.065	II	797	I

Table 1. (Cont'd.)

PI Number	Maturity Group	PI Number	Maturity Group	PI Number	Maturity Group
88.798	II	89.167	II	91.725-3	II
803	II	170	II	732-1	I
804	I	171	II	732-2	I
805-2	I	90.180	II	733	I
805-4	II	560	II	92.109	II
810	II	567	I	460	II
825	II	570	II	464	II
997	II	574	II	465	II
89.000	II	90.575	II	468	I
001	O	91.091	II	469	I
89.003-1	II	91.102	II	92.470	I
004	II	104	II	561	II
005-5	II	107	II	563	II
006-2	II	109	II	565	I
008	II	110	I	569	II
012	II	110-1	I	570	II
013	II	114	II	571	II
014	II	115	II	572	II
053	II	116	II	573	II
055	I	117	II	576	II
89.055-1	II	91.119	II	92.580	II
056-3	I	120	II	582	II
057	I	120-2	II	583	II
058	I	123	I	589	II
059	II	124	II	592	II
060	I	126	II	595	II
061-1	I	129	II	596	II
063	II	132-2	II	598	II
064	II	138	II	603	II
065	II	141	II	607	II
89.065-2	II	91.144	II	92.611	II
070	II	150	II	625	I
072	II	156	II	627	II
075	II	161	II	629	II
138	II	167	II	633	II
153	II	171	II	639	II
154	II	180	II	649	I
154-1	II	557	II	660	II
156	II	559	II	661	II
92.671	II	135.590	II	153.252	00
677	II	142.491	I	253	I
681	II	151.249	00	255	I
683	II	152.361	O	261	O

Table 1. (Cont'd.)

PI Number	Maturity Group	PI Number	Maturity Group	PI Number	Maturity Group
684	II	573	00	262	0
687	II	153.203	00	263	I
694	II	208	00	264	II
698	II	209	0	265	0
705	II	210	00	266	I
		211	00	267	I
92.706	I	153.212	00	153.270	0
717	II	213	0	271	I
719	II	214	I	272	0
733	II	215	I	273	0
734	II	216	00	274	I
748	II	217	00	275	0
93.217	II	218	00	276	I
559	II	219	00	277	0
560	II	221	00	278	0
565	II	222	00	279	I
96.152	I	153.225	00	153.280	II
171	II	226	II	281	0
188	II	227	I	282	I
193	I	229	I	283	I
195	II	230	00	284	0
201	II	233	0	285	I
549	II	234	00	286	0
97.605	II	235	0	287	I
103.414	II	236	I	288	II
131.531	I	237	0	289	II
132.201	I	153.238	I	153.290	I
203	00	240	0	291	I
204	0	241	0	294	I
205	0	242	0	297	00
206	I	244	I	299	00
207	0	245	0	300	0
214	00	246	0	301	00
215	I	247	I	302	00
217	00	249	0	303	00
135.589	II	250	I	304	0
153.305	I	181.532	I		
306	0	533	II		
308	I	534	II		
310	I	536	I		
311	I	538	I		
313	I	541	II		
314	0				

Table 1. (Cont'd.)

PI Number	Maturity Group
315	I
316	I
317	O
153.318	O
319	O
320	O
154.189	O
190	OO
191	O
192	O
193	OO
194	O
195	II
154.196	O
198	OO
199	OO
200	OO
159.764	OO
161.431A	OO
988	OO
989	O
171.421	I
180.501	O
180.502	OO
507	OO
508	OO
509	OO
516	OO
517	OO
524	OO
529	O
530	O
532	I

Table 2. Plant introductions (PI) of wild soybeans (Glycine soja) screened for resistance to stem rot by the excised stem assay.

PI Number	Maturity Group	PI Number	Maturity Group	PI Number	Maturity Group
65.549	II	423.995	O	464.925A	O
81.762	II	996	OO	B	O
101.404A	II	997	OO	C	I
B	II	998	OO	928	OO
135.624	II	999A	OO	929A	O
326.581	II	B	OO	B	O
582A	II	424.000	OO	468.904	OO
B	I	001	OO	905	O
342.618A	II	002	OO	906	O
B	I	003	OO	907	I
342.619A	O	424.004A	II	468.909	OO
B	OO	440.913A	II	910	OO
620A	I	B	II	911	OO
B	II	447.003A	O	912	OO
621A	OO	B	I	913	OO
B	OO	458.535	O	479.745	I
C	OO	536	O	746A	II
622A	I	539B	O	746B	II
B	I	540A	O	748	II
391.587	II	B	O	750	I
407.288	II	458.540C	O	479.752	I
289	II	D	O	753A	II
290	II	464.866A	OO	B	II
291	II	B	OO	767	I
292	II	868A	OO	768	O
293	II	B	OO	769	O
294	II	869A	O	483.459	I
295	II	B	O	461	II
296	II	871A	O		
297	II	B	O		
407.298	II	464.871C	O		
299	II	889A	II		
423.988	OO	B	II		
989A	OO	C	II		
B	O	890A	II		
990A	O	B	I		
B	O	891A	I		
991	O	B	II		
992	OO	C	II		
994	O	892	I		

Table 3. Soybean introductions having stem rot lesions shorter than those of Corsoy in one or more tests.

PI No.	Lesion lengths					
	Test 1		Test 2		Test 3	
	cm	% of Corsoy	cm	% of Corsoy	cm	% of Corsoy
54.606-2	10.6	97.7	2.7*	38.6	3.4	105.9
54.608-2	9.9	91.9	4.4*	62.8	9.9	167.3
54.855	5.1*	48.1	4.4	73.9	11.9	105.7
68.011	4.8	281.1	9.8*	87.1	9.9	94.7
68.708	7.4*	57.9	10.0	97.3	12.8	110.8
70.243	4.0	229.5	6.5*	61.3	10.4	175.4
79.737	3.8*	48.3	10.4	140.9		
79.739	11.4*	84.2	9.1	124.0		
81.037-4	5.7	116.3	6.8*	73.2		
81.777	9.3*	66.8	11.9	129.4		
82.263-1	7.6	85.8	7.7*	73.1	9.4	158.7
82.263-3	5.3	37.9*	12.5	118.2		
82.527	6.0	43.3*	12.2	10.6		
82.534	3.1	22.5*	11.5	109.0	8.0	134.9
83.889	9.0	85.3	4.5*	32.6		
83.891	8.6*	61.7	10.9	103.4		
83.923	9.9*	70.9	9.8	93.2		
84.637	7.7*	74.9	4.4	96.5		
84.666-1	5.6*	54.5	3.8	83.9		
84.750	5.2	51.1	5.3	117.4		
85.625	5.5*	56.8	5.0	109.9		
86.021	4.7*	48.8	3.1	68.0		
89.001	8.0	146.9	0.4*	9.3		
89.058	8.1*	84.9	1.4	30.9		
92.687	3.5	34.0*	2.4	173.4		
153.214	7.6	90.6	4.9*	41.5		
153.233	3.3	102.4	4.2*	40.5	3.1	220.9
153.242	7.7*	74.1	0.1*	5.7		
153.246	4.7*	45.3	2.6	189.9		
153.265	2.9	87.8	3.0*	25.9		
154.191	5.5*	52.7	1.0	74.1		
154.193	6.6*	52.7	2.7	194.2		
153.194	3.9*	37.9	0.5	33.1		
159.764	3.7*	68.1	4.7	144.7		
171.421	4.1*	39.5	4.5	323.7		

*=Lesion length significantly shorter than that of Corsoy by LSD test ($P=0.05$).

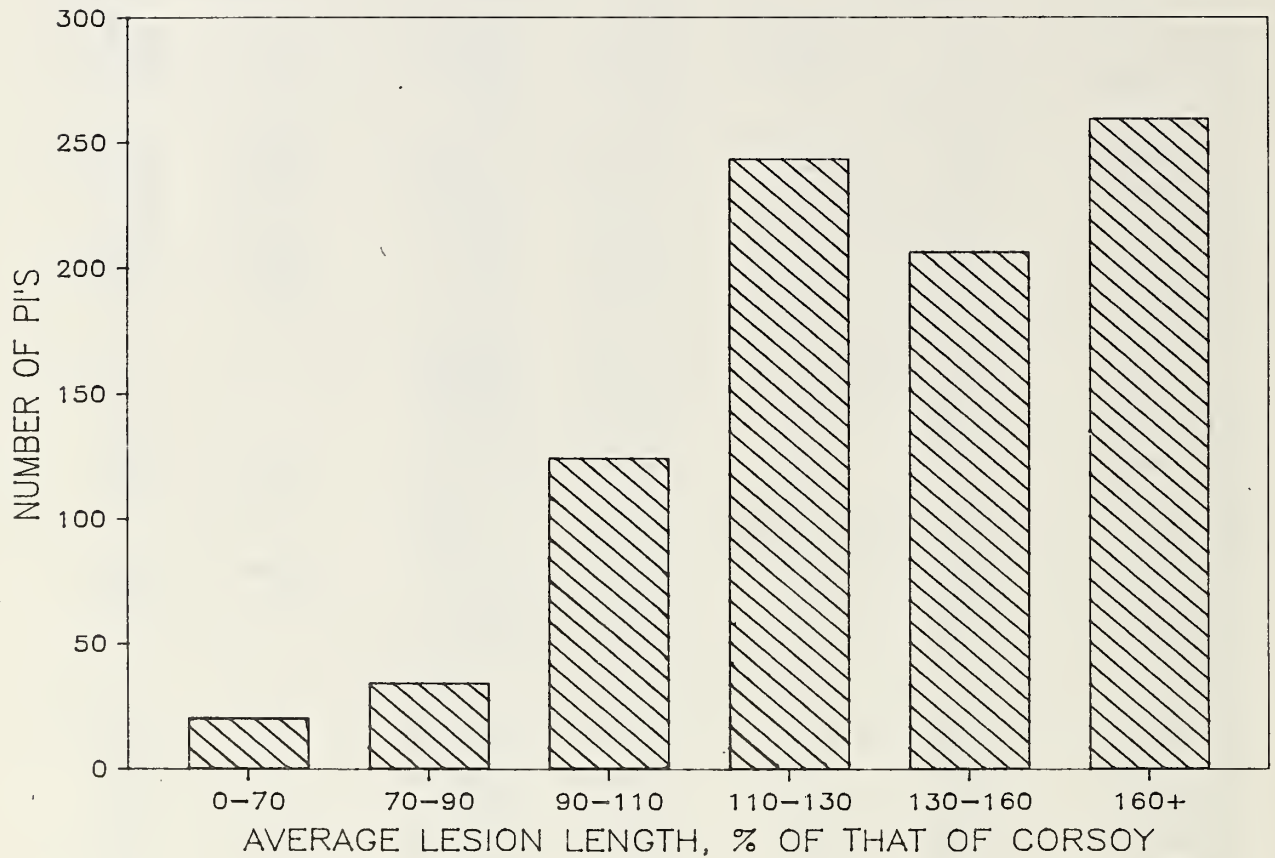


Figure 1. Frequency distribution of stem rot lesion lengths of 886 soybean (*Glycine max*) introductions (PI's) using the excised stem laboratory assay. Values represent results of a single test for 740 PI's and the means of two for more tests for 146 P.I.'s.

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1) Evaluation of Minnesota-adapted soybean germplasm for frequency of plant regeneration via organogenesis and investigations of Agrobacterium-mediated transformation.

One method of gene transfer utilizes the natural ability of Agrobacterium tumefaciens, an infectious soil bacterium responsible for crown gall disease in many dicotyledonous plants, to introduce foreign DNA into plant cells. Transformed antibiotic-resistant soybean callus was obtained from Agrobacterium-mediated transformation (Facciotti et al., 1985; Byrne et al., 1987). Transformed cells may be regenerated via tissue culture to form transgenic plants. Germline transformation of regenerated plants using Agrobacterium as the vector and subsequent Mendelian segregation of the marker genes in the progeny has been reported (Hinchee et al., 1988). To date no soybean lines adapted to Minnesota have been genetically engineered via Agrobacterium.

When using Agrobacterium-mediated transformation, the frequency of obtaining a transgenic soybean plant is predominantly the product of frequencies of two events. These two events are the frequency of transformation of totipotent cells and the frequency of plant regeneration from totipotent cells. The objectives of this study were to 1) evaluate these soybean lines for frequency of plant regeneration via organogenesis (Wright et al., 1986) in tissue culture and 2) use the most responsive genotype in an Agrobacterium-mediated transformation and regeneration experiment.

Materials and methods: Evaluation of Regeneration--Ten soybean lines adapted to Minnesota were evaluated for their ability to regenerate in tissue culture. The lines were 'Corsoy 79', 'Evans', 'Gnome 85'; 'Hodgson 78', 'Maple Amber', 'McCall', 'Sibley', Experimental Line HHP, PI 180529, and PI 445799. Sterile mature seeds were germinated on 1/2MSBA medium, which contains 5 uM benzyladenine (Wright et al., 1986). All media in this protocol were supplemented with 0.17 uM $\text{Ni}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ to provide sufficient amounts of nickel, an essential element (Eskew, 1983). The cotyledonary node was excised after 14 days and placed basipetally on fresh media. Shoots (approx. 1 cm) primarily adventitious, were removed every 2-4 weeks and placed on the respective media to allow formation of roots. Developed plantlets with several trifoliolates and roots were hardened off and grown to maturity in the greenhouse, or were discarded, but regarded as mature plants since virtually all plantlets developed into mature plants.

Two experiments with eight seeds of each of the ten selected soybean lines were germinated, explanted, and evaluated. Initially, four cotyledonary nodes were cultured on each petri plate. Observations of the number of shoots and the number of regenerated plantlets per cotyledonary node were averaged within each initial plate. Each plate served as an experimental unit and the experiment was analyzed as a completely randomized design. The soybean line variable was a fixed effect. Comparisons between overall means of number of plants regenerated per soybean line were made using a protected LSD.

Transformation Experiments--The pMP90/pBI121 Agrobacterium binary vector, a disarmed C58 strain that transfers the neomycin phosphotransferase (NPTII) and B-glucuronidase (GUS) genes, was suspended in liquid 1/2MSBA medium containing 20 μ M acetosyringone. Acetosyringone is known to be a virulence-inducing compound in soybean transformation (Owens and Smigocki, 1988). The same vector without the pBI121 binary plasmid, designated pMP90, served as a control. The cotyledonary node region of 6-8 d Hodgson 78 and Peking seedlings germinated aseptically on 1/2MSBA medium were wounded and infected with the bacterial inoculum, using a syringe with a 25-gauge needle. Further culture of inoculated cotyledonary nodes was by the same organogenic culture procedure (Wright et al., 1986) with the addition of surface-applied 500 μ g/ml cefotaxime sulfate and the presence or absence of surface-applied kanamycin (50 μ g/ml) or G418 (10 μ g/ml). Portions of all shoots produced over a period of 40 weeks were assayed for fluorometric GUS activity. Most GUS-positive shoots were streaked on a bacterial plate to check for bacterial contamination, then rooted and grown to maturity in a controlled growth chamber. Leaves from ca. 5 internodes of the maturing plants were assayed for fluorometric GUS activity to determine if the plants were transgenic throughout, chimeric, or escapes.

Results and discussion: All soybean lines produced adventitious shoots and regenerated plants (Table 1). The mean number of shoots and regenerated plants from all soybean genotypes was 23.22 and 6.16 per cotyledonary node, respectively, yielding a mean shoot-to-plant conversion frequency of 26.4%. Although shoot formation frequency did not differ significantly for lines, the number of regenerated plants from each line differed significantly ($p < 0.01$). The soybean lines Experimental Line . HHP, Evans, PI 445799, Hodgson 78, Corsoy 79, and PI 180529 produced the most regenerated plants per cotyledonary node. Approximately twice as many shoots per cotyledonary node were obtained with 'Wayne', a maturity group III line, (Wright et al., 1986), than with the best responding Minnesota-adapted soybean lines evaluated. Wright et al. (1986) reported that soybean lines of all maturity groups responded favorably to this type of regeneration. This suggests that either culture techniques or genotype differences may contribute to changes in regeneration frequency between laboratories.

Hodgson 78 was the Minnesota-adapted soybean line selected for the

subsequent transformation experiments. This line was chosen because it regenerated plants at a high frequency and it is susceptible to Agrobacterium infection. Peking, a maturity group III line, was also used in the transformation experiments because it is very susceptible to Agrobacterium infection (Owens and Cress, 1985; Byrne et al., 1987).

Regenerating shoots arising from cotyledonary nodes that had been wounded and inoculated with the binary vector pMP90/pBI121 and control were isolated and assayed for fluorometric GUS activity. Some cultures inoculated with pMP90/pBI121 formed shoots that gave a false positive reaction to the fluorometric GUS assay. False positive shoots were defined as shoots that initially tested positive for GUS activity, but tested negative at a later stage. Most shoots that tested positive were streaked on bacterial plates and revealed persistent Agrobacterium growth. This provides some evidence that the source of GUS activity was bacteria-derived. The Agrobacterium vector presumably has readthrough transcription of the lacZ transcript in the pBI121 plasmid, allowing bacteria-derived GUS activity (Jefferson, 1987). Most positive shoots were rooted and grown to maturity in a growth chamber to allow the assay of additional nodes in an environment less conducive to bacterial growth. These plants, when later tested, were negative for GUS activity at all nodes sampled, indicating that transformation was not responsible for the GUS activity.

A Peking callus line that exhibited GUS activity was isolated from an inoculated cotyledonary node placed on kanamycin-selection medium. This cell line was not contaminated with any bacterium, suggesting that the GUS activity did not come from an exogenous source. Histochemical GUS assays showed that the GUS activity was inside the cells, providing further evidence of an endogenous source of expression, suggesting that this callus was transformed. However, this callus did not exhibit indisputable kanamycin resistance.

The soybean transformation experiments did not result in transgenic soybean plants, indicating that improvements are necessary. Improvements in the transformation vector and in the tissue culture selection of transgenic tissue may result in effective transformation of Minnesota-adapted soybeans.

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Table 1. The mean number of shoots formed and plants regenerated per cotyledonary node explant on 1/2MSBA organogenic medium.

Soybean line	Shoots per cotyledonary node	Plants per cotyledonary node	Conversion (%)
Exp. Line HHP	31.5	9.1 a	28.7
Evans	30.3	8.1 ab	26.6
PI 445799	23.8	8.0 ab	33.6
Hodgson 78	19.4	7.9 ab	40.8
Corsoy 79	22.8	7.8 ab	34.2
PI 180529	30.3	6.7 abc	22.1
McCall	18.9	5.4 bcd	28.8
Sibley	23.3	3.6 cd	15.5
Gnome 85	20.4	2.8 d	13.5
Maple Amber	11.4	2.2 d	19.4
Average	23.2	6.2	26.4
LSD (0.05)	NS	3.6	

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1) Linkage for resistance to race 5 and race 3 of the soybean cyst nematode (*Heterodera glycines*) in the cross PI 437654 x Tracy M.

The soybean cyst nematode (SCN) was discovered in the USA in 1954 by Winstead et al. (1955). SCN populations were soon found to be heterogeneous and were divided into races by Golden et al. (1970). Studies to date have investigated the inheritance of resistance to SCN to each race independently. However, Triantaphyllou (1975) stated that race 3 had no genes for parasitism of resistant cultivars. Therefore, resistance to race 3 would appear a prerequisite for resistance to any of the other races. This is further supported by the studies of Anand et al. (1985, 1988) who found all lines resistant to race 4 or 5 were also resistant to race 3. To confirm this theory, an experiment was initiated to study segregation in an F2 population of the cross PI 437654 x Tracy M for races 3 and 5.

Materials and methods: PI 437654 (resistant to both races 3 and 5) was crossed with the cultivar 'Tracy M' (susceptible to races 3 and 5) during the summer of 1986. The F1 plants were grown at the Lee farm of the University of Missouri, Portageville, MO in the summer of 1987. The F2 population along with 'Essex', 'Peking', PI 88788, and PI 90763 were tested against races 3 and 5 of SCN. The seeds were germinated on germination paper. When the radical was approximately 3 cm long, the primary root meristem was removed. This stimulated lateral root branching. After 6 days, the roots of each plant were divided and placed in adjacent 250-ml styrofoam cups held together by duct tape. The cups were filled with steam-sterilized soil. Five days later, one side of the plant root was inoculated with race 3 and the other side with race 5. Each cup received approximately 1000 eggs of either race. Thirty-two days after the inoculation, plants were soaked in water for easy removal from the cups. Lateral roots were clipped from the tap root, rinsed in clean water, lightly blotted, and weighed. The plants with less than 0.25 g of root were discarded. Roots were then placed in a 100-mesh screen nested in a 60-mesh screen. Cysts were dislodged from the roots with a water jet and collected on the lower screen. Following this procedure, cysts were enumerated under a microscope. An index of parasitism (IP) was calculated as the number of cysts on a plant / the number of cysts on Essex x 100. An IP of 10 or more was taken to be a susceptible reaction, while those having an IP of less than 10 were considered resistant (Golden et al., 1970).

Results and discussion: The cyst counts and the index of parasitism are summarized in Table 1. Populations from the crosses PI 437654 x Tracy

M segregated for SCN reaction in the F₂ generation. Of the 105 F₂ plants tested, 100 were resistant to race 3 and four were resistant to race 5. Three plants were resistant to both races 3 and 5, whereas only one plant has been observed that is resistant to race 5 and susceptible to race 3. It is possible that such an error resulted from the labels on the cups being exchanged. If this plant is ignored, the data could be explained by three recessive genes conditioning resistance to race 5. Two of these genes condition resistance to race 3, with an additional recessive gene required for resistance to race 5. Since a plant with resistance to race 5 and susceptible to race 3 has been found, an alternate explanation would be that one recessive gene (a) is required for resistance to either race 3 or 5. A second recessive gene (b) is required for resistance to race 3. This gene must be linked to a recessive gene (c) conditioning resistance to race 5. A fourth gene (d) at an independent locus would also be required for resistance to race 5. This could result in the following reactions:

A_ _ _ susceptible to both races 3 and 5
 aa bb _ _ D_ resistant to race 3 and susceptible to race 5
 aa bb C_ _ _ resistant to race 3 and susceptible to race 5
 aa B_ cc dd susceptible to race 3 and resistant to race 5

It may be concluded that resistance to races 3 and 5 in the cross PI 437654 x Tracy M is not inherited independently. It is likely that the genes required for resistance to race 3 are a subset of those required for resistance to race 5 or that some of the genes for resistance to races 3 and 5 are closely linked.

Table 1. Reaction of checks and F₂ plants to soybean cyst nematode races 3 and 5.

Entry	Race	Mean	Range	Number of plants	
				R	S
Essex	3	109.8	40-201	0	8
	5	186.3	71-283	0	8
88788	3	0.5	0-1	4	0
	5	73.0	35-155	0	4
90763	3	3.0	0-14	4	1
	5	0.3	0.2	5	0
Peking	3	0.1	0.1	5	0
	5	6.4	0.16	8	0
F ₂	3	74.0	1-209	10	95
	5	146.8	1-405	4	101
	3 & 5			3	102

R = resistant; S = susceptible

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 1) Testing for genetic linkage of morphological and electrophoretic loci in the cultivated soybean

Combining information from electrophoretic and whole plant analyses should enhance the development of the genetic linkage map for soybean (Devine et al., 1984; Kiang and Chiang, 1987). Seventeen of the 20 possible linkage groups in soybeans have been identified (Bult et al., 1989). However, each of these linkage groups has only a few markers (Kiang and Chiang, 1987) and only one locus (Dial) has been assigned unambiguously to a chromosome (chromosome D) (Hedges and Palmer, 1986). Many more markers are needed to help distinguish linkage groups and for selective breeding for qualitative and quantitative characters (Tanksley, 1983).

Soybean cultivars AV68 and T289 were crossed to study linkage relationships among electrophoretic and morphological loci. Eight electrophoretic loci (Aco2, Adhl, Dial, Got, Idhl, Mpi, Pgdl, Pgml) and three morphological traits (nodulation response, hypocotyl color, and pubescence color) segregated in the F2. The genotypes of the parental lines are shown in Table 1.

F2 seed from AV68 x T289 and its reciprocal were harvested from greenhouse-grown F1 plants. A small piece of cotyledon, cut opposite the plumule end, was removed from each F2 seed for electrophoresis. Detailed procedures for gel electrophoresis and for enzyme activity staining are described elsewhere (Gorman, 1983; Devine et al., 1984; Griffin and Palmer, 1987; Kiang et al., 1987). The remaining part of the seed was sent to Beltsville, MD, for analysis of whole plant characteristics.

The Rj2 allele in soybean conditions an ineffective nodulation response with strains USDA 7, USDA 14, and USDA 122 of Bradyrhizobium japonicum (Caldwell, 1966). The ineffective nodulation response is characterized by the presence of cortical nodulation and/or small nodular-like structures on the roots instead of normal nodules. Plants homozygous for the recessive rj2 allele form normal root nodules with rhizobial strains USDA 7, 14, and 122. Plants carrying the Rj2 allele cannot nodulate normally with these strains.

Seeds were planted in sterile vermiculite in growth trays (Devine and Reisinger, 1978) and inoculated with a broth culture of strain USDA 7 that elicits the nodulation responses defining the Rj2 versus rj2 alleles. The seeds then were covered with one-half inch of perlite as a barrier to

contamination by rhizobia from the air. After 17-20 days of growth, the seedlings were scored for green versus purple hypocotyl, extracted from the vermiculite and scored for nodulation response. Each seedling then was placed in a test tube containing water and transplanted, with a labeled stake, to the field. After three and a half months of growth in the field, the plants were scored for pubescence color, tawny (T-) versus gray (tt).

Single-gene F2 segregation data for isozyme variants and morphological traits are shown in Table 2. Segregation of alleles at the Got locus did not conform to the expected 1:2:1 Mendelian ratio. We suspect this was due to the difficulty of distinguishing the fast mobility variant (Got-c/-c) from the heterozygote (Got-b/-c). Hypocotyl color and nodulation response also did not conform to the expected Mendelian ratios (3:1). For hypocotyl color, an excess of green hypocotyls was detected. This may have been due to environmental effects (temperature and/or light conditions in the greenhouse) masking the expression of anthocyanin in the plant tissues. The deviation from the expected 3:1 ratio for nodulation response may have been due to chance.

Tests of independent assortment of gene pairs were performed as described by Foster and Rutger (1978). Results of the analysis are shown in Table 3. Only the degrees of freedom due to linkage are reported. When the data deviated significantly from the expected ratio, recombination frequency was estimated, using the Pascal computer program, QUIKLINK (Suiter et al., 1983). Linkage was detected between Adhl and Wl ($r=29.8 \pm 3.0\%$). This linkage was reported previously by Kiang and Chiang (1987) with a recombination frequency of $20.6 \pm 1.2\%$. The discrepancy between the two values is most likely due to error. No conclusive evidence of linkage was detected between any of the other loci tested. As has been noted in studies similar to the one reported here (Devine et al., 1983, 1984; Kiang and Chiang, 1985), it is important to report instances of independent assortment as well as genetic linkage to aid in the ability to distinguish linkage groups and to prevent redundant analyses.

Table 1. Genotypes of the parental lines used in this study.

Line	Genotypes									
AV 68	<u>Aco2-a</u>	<u>Adhl</u>	<u>Dial</u>	<u>Got-b</u>	<u>Idhl-a</u>	<u>Mpi-c</u>	<u>Pgd1-a</u>	<u>Pgml-a</u>	<u>rj2</u>	<u>Wl T</u>
	<u>Aco2-a</u>	<u>Adhl</u>	<u>Dial</u>	<u>Got-b</u>	<u>Idhl-a</u>	<u>Mpi-c</u>	<u>Pgd1-a</u>	<u>Pgml-a</u>	<u>rj2</u>	<u>Wl T</u>
T289	<u>Aco2-b</u>	<u>adh1</u>	<u>dial</u>	<u>Got-c</u>	<u>Idhl-b</u>	<u>Mpi-b</u>	<u>Pgd1-b</u>	<u>Pgml-b</u>	<u>Rj2</u>	<u>wl t</u>
	<u>Acp2-b</u>	<u>adh1</u>	<u>dial</u>	<u>Got-c</u>	<u>Idhl-b</u>	<u>Mpi-b</u>	<u>Pgd1-b</u>	<u>Pgml-b</u>	<u>Rj2</u>	<u>wl t</u>

Table 2. Single locus F2 segregation data.[†]

Locus	No. in each genotypic class [‡] N				χ^2 [§]	d.f.	P	
	x/x	x/y	y/y					
<u>Aco2</u>	244	476	231	951	0.36	2	0.8	<p< 0.9
<u>Dial</u>	266	494	254	1014	0.95	2	0.5	<p< 0.7
<u>Got</u>	257	533	218	1008	6.36*	2	0.025	<p< 0.05
<u>Idh1</u>	228	508	247	983	1.84	2	0.1	<p< 0.5
<u>Mpi</u>	223	498	261	982	3.14	2	0.1	<p< 0.5
<u>Pgdl</u>	241	539	231	1011	4.64	2	0.1	<p< 0.5
<u>Pgml</u>	206	399	212	817	0.53	2	0.1	<p< 0.5
	<u>X</u>	<u>xx</u>						
<u>Adh1</u>	740	249		989	0.016	1	0.9	<p< 0.98
<u>Rj2</u>	723	205		928	4.19*	1	0.025	<p< 0.05
<u>W1</u>	677	308		985	20.65**	1		<p< 0.0001
<u>T</u>	686	216		902	0.65	1	0.3	<p< 0.5

[†]Reciprocal crosses combined.[‡]x and y represent alternate alleles at a locus.[§] χ^2 goodness-of-fit test for 1:2:1 or 3:1 expected ratios

* Significant at the 0.05 level.

** Significant at the 0.0001 level.

Table 3. F2 segregation data from soybean dihybrids from the cross AV68 x T289 and its reciprocal for linkage analysis.

Gene pair	Segregation†						N	d.f	χ^2 †	P	%R±S.E.
	$\frac{AB}{AB}$	$\frac{AB}{aB}$	$\frac{AB}{Ab}$	$\frac{AB}{aB} + \frac{AB}{Ab}$	$\frac{Ab}{Ab}$	$\frac{aB}{aB}$					
Aco2-Dial	58	132	137	217	48	127	948	4	7.89*	0.10	49.5 ± 1.6
Aco2-Got	53	140	125	245	63	90	945	4	9.93*	0.04	47.8 ± 1.6§
Aco2-Idh1	65	108	117	228	55	121	917	4	4.05	0.40	47.7 ± 1.6
Aco2-Mpi	55	106	112	233	68	120	917	4	0.76	0.94	49.5 ± 1.6
Aco2-Pgd1	54	126	134	251	56	99	951	4	5.63	0.23	48.6 ± 1.6
Aco2-Pgm1	54	86	88	210	54	107	802	4	7.62	0.11	48.2 ± 1.8
Dial-Got	63	129	145	254	57	107	1006	4	0.72	0.95	49.5 ± 1.6
Dial-Idh1	58	111	140	254	61	112	982	4	4.80	0.31	48.8 ± 1.6
Dial-Mpi	62	114	130	243	65	122	979	4	3.44	0.49	47.6 ± 1.6
Dial-Pgd1	74	109	130	274	61	107	1008	4	4.73	0.32	48.1 ± 1.6
Dial-Pgm1	59	103	103	190	52	109	814	4	2.44	0.65	48.5 ± 1.8
Got-Idh1	47	127	137	268	66	121	977	4	4.96	0.29	48.2 ± 1.6
Got-Mpi	58	113	117	270	73	128	971	4	2.79	0.59	49.4 ± 1.6
Got-Pgd1	63	123	125	285	64	122	998	4	2.04	0.73	48.7 ± 1.6
Got-Pgm1	54	113	109	204	50	115	811	4	1.68*	0.79	48.6 ± 1.8
Idh1-Mpi	38	116	110	253	74	124	952	4	11.01*	0.03	44.8 ± 1.6§
Idh1-Pgd1	43	130	131	251	53	122	977	4	5.66	0.23	47.9 ± 1.6
Idh1-Pgm1	45	111	92	196	55	105	797	4	2.83	0.59	49.3 ± 1.8
Mpi-Pgd1	56	104	107	279	60	109	976	4	7.30	0.12	47.4 ± 1.6
Mpi-Pgm1	40	101	92	190	45	106	785	4	1.20	0.89	48.9 ± 1.8
Pgd1-Pgm1	47	113	97	204	53	111	817	4	0.96	0.92	49.4 ± 1.7

Table 3. (continued)

Gene pair	Segregation				N	d.f.	χ^2	P	%R \pm S.E.
	$\frac{AB}{A_}$	$\frac{AB}{a_}$	$\frac{aB}{a_}$	$\frac{Ab}{Ab}$					
<u>Aco2-Adh1</u>	172	357	165	62	922	2	2.01	0.37	49.8 \pm 2.0 ^s
<u>Aco2-Rj2</u>	166	334	175	66	876	2	6.76*	0.03	44.6 \pm 2.0
<u>Aco2-w1</u>	158	321	156	75	920	2	0.22	0.89	49.1 \pm 2.0
<u>Aco2-I</u>	161	321	163	51	839	2	0.56	0.76	48.6 \pm 2.0
<u>Adh1-Dial</u>	193	366	177	65	985	2	3.05	0.21	48.0 \pm 2.0
<u>Adh1-Got</u>	192	382	159	58	980	2	1.12	0.57	49.4 \pm 2.0
<u>Adh1-Idh1</u>	168	367	181	53	954	2	0.84	0.66	49.6 \pm 2.0
<u>Adh1-Mpi</u>	165	356	190	50	953	2	1.26	0.53	49.6 \pm 2.0
<u>Adh1-Pgd1</u>	170	400	165	66	982	2	2.59	0.27	49.4 \pm 2.0
<u>Adh1-Pgm1</u>	151	301	163	55	814	2	0.91	0.63	47.9 \pm 2.0
<u>Dial-Rj2</u>	192	345	183	49	924	2	0.58	0.75	48.6 \pm 2.0
<u>Dial-w1</u>	187	328	159	73	981	2	4.11	0.13	46.6 \pm 2.0
<u>Dial-I</u>	182	332	171	52	898	2	0.50	0.78	48.6 \pm 2.0
<u>Got-Rj2</u>	193	387	138	47	919	2	5.64	0.06	45.1 \pm 2.0
<u>Got-w1</u>	175	363	135	72	976	2	3.55	0.17	47.4 \pm 2.0
<u>Got-I</u>	175	354	152	57	893	2	1.48	0.48	48.1 \pm 2.0
<u>Idh1-Rj2</u>	162	373	166	43	896	2	1.01	0.60	48.3 \pm 2.0
<u>Idh1-w1</u>	149	351	156	74	951	2	1.79	0.41	49.9 \pm 2.0
<u>Idh1-I</u>	156	342	167	45	869	2	0.56	0.76	49.9 \pm 2.0
<u>Mpi-Rj2</u>	164	358	176	37	893	2	3.19	0.20	46.4 \pm 2.0
<u>Mpi-w1</u>	154	324	168	64	989	2	0.86	0.65	48.2 \pm 2.0
<u>Mpi-I</u>	151	336	174	47	870	2	0.84	0.66	48.9 \pm 2.0 ^s
<u>Pgd1-Rj2</u>	171	370	177	49	921	2	6.03*	0.05	46.9 \pm 2.0 ^s
<u>Pgd1-w1</u>	166	359	147	66	978	2	1.68	0.43	47.7 \pm 2.0
<u>Pgd1-I</u>	169	350	163	45	895	2	2.71	0.26	49.6 \pm 2.0
<u>Pgm1-Rj2</u>	149	279	143	40	737	2	0.27	0.87	48.9 \pm 2.0
<u>Pgm1-w1</u>	139	277	128	63	791	2	4.17	0.12	47.6 \pm 2.0
<u>Pgm1-I</u>	137	278	137	45	714	2	0.91	0.63	49.3 \pm 2.0

Table 3. (continued)

Gene pair	Segregation			N	d.f.	χ^2	P	%R \pm S.E.
	$\frac{AB}{--}$	$\frac{Ab}{b}$	$\frac{aB}{a}$	$\frac{ab}{ab}$				
Adh1-Rj2	513	155	188	46	902	1	1.26	46.9 \pm 2.6 [†]
Adh1-W1	556	157	102	142	957	1	110.75 ^{**}	29.8 \pm 3.0
Adh1-I	488	160	179	49	876	1	0.95	47.4 \pm 2.6
Rj2-W1	513	209	136	69	927	1	1.69	47.5 \pm 2.5
Rj2-I	536	166	146	49	897	1	0.18	48.7 \pm 2.0
W1-I	482	151	204	65	902	1	0.01	49.9 \pm 2.5

[†]A represents the first locus and B the second locus of the gene pairs, respectively.

[‡] χ^2 goodness-of-fit test based on a 1:2:2:4:1:2:1:2:1, 3:6:3:1:2:1 or 9:3:3:1 ratio.

[§]Simulated repulsion.

[¶]Coupling

*Significant at the 0.05 level.

**Significant at the 0.00001 level.

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2) Electrophoretic methods for soybean genetics studies.

Detecting different enzyme forms via gel electrophoresis has proved valuable for soybean cultivar identification, parentage analysis, population and genetic studies, and genome mapping (Kiang and Gorman, 1983).

This article is a compilation of the electrophoretic procedures used in our laboratory. It includes details on sample preparation, buffer formulations, gel preparation, and enzyme-activity staining protocols. We encourage and appreciate readers' ideas and comments on the techniques described here, as well as information on additional enzyme-activity assays.

Gel Types: We use three types of electrophoretic gels: acrylamide, starch, and a mixture of the two. By using the different kinds, combinations and concentrations of materials, we can change gel porosity and enhance the resolution of enzyme-activity banding patterns. Cofactors, NADP and NAD, are added to some of the gels to increase staining intensity of the bands. The acrylamide/starch concentrations we use most often are: 12.5% starch, 7% acrylamide, 9% acrylamide, 7% acrylamide + 2% starch, and 6% acrylamide + 4% starch. For gels containing acrylamide, we prepare the total amount of gelling agent as 95% acrylamide and 5% N,N'-methylene-bis-acrylamide. Gel polymerization catalysts, ammonium persulfate (APS) and N,N,N',N'-tetramethylethylene diamine (TEMED), are 0.1% (w/v) and 0.2% (v/v) of the total volume of the gel buffer, respectively.

We use gel molds made from PVC (polyvinylchlorine) board. We order the raw materials from a local supplier and assemble the molds ourselves. The three dimensions (length x width x depth) we use routinely are: 20.5 x 17.8 x 0.3 cm (one layer, holds 150 ml buffer), 20.5 x 17.8 x 0.6 cm (two layer, holds 210 ml buffer) and 20.5 x 17.8 x 0.9 cm (three layer, holds 280 ml buffer). By using the deeper molds, we can remove multiple gel slices and stain for activity of different enzymes having similar electrophoretic mobilities, thereby conserving materials, time and space.

Sample Preparation: Although almost any tissue can be used with our methods, we generally use a small piece of cotyledon, cut opposite the plumule, from each seed. In this way, the genotype of the plant can be determined without affecting seed germinability.

Remove the seed coat from the cut portion of the seed and place the dry cotyledon tissue in a grinding well. Our sample grinding trays consist of several rows of shallow, round wells that we have drilled into PVC board. Soak the tissue in 2-4 drops (depending on the amount of tissue) of 0.005 M L-histidine (HCl) (pH 7.0) buffer for at least 8 hours. Then grind the tissue well, using glass rods with rounded smooth ends. Place a 1 x 1 cm square of lens paper on top of each well to serve as a filter between the homogenate and the wick. Absorb the homogenized tissue samples onto wicks cut from bibulous paper. The size of the wick depends on gel thickness and sample number per gel.

We have found that this method of sample preparation works well for a large number of enzymes, except urease. To screen for urease isozyme variants, soak the tissue in a 60 mM Tris-HCl (pH 8.2) extraction buffer with 15 mM CaCl_2 , 390 mM sucrose and 10 mM dithiothreitol (DTT) (Kloth et al., 1988). Add the DTT just before use and grind the seeds immediately.

Centrifugation of the samples (10 min at 5,000X g at 4 C) can enhance resolution of some of the enzyme-activity bands, although sufficient resolution is achieved in most cases without this step.

Loading the Gel: Hold a scalpel vertically against a straightedge approximately 3 cm from the edge of the gel mold and draw across and through the gel to form a sample well. Alternatively, individual wells can be made by inserting into the gel a ruler to which 26 razor blade fragments (1 x 0.5 cm) are attached and arranged evenly, like teeth on a comb. Using forceps, remove one wick at a time from the sample wells, blot it lightly on an absorbent tissue and then insert it perpendicularly into the gel slit. Place the wicks evenly along the gel, using the markings on the straightedge as guides. Leave at least a 0.5 cm space on either end of the line of wicks to eliminate edge effects. When all the wicks have been inserted, lightly press the two sections of gel back together along the suture line to remove air gaps and to ensure good contact between the wicks and the gel.

Electrophoresis: Rubbermaid plastic trays (22.5 x 8 x 5 cm) can serve as electrode buffer reservoirs. To modify the trays, carefully drill a small hole into one end of the tray, approximately 1 cm from the top. Fit a male portion of a banana plug into the hole. Wind one end of a 22-cm length of thin platinum around the inside part of the plug once or twice and then place the remaining length of wire along the bottom of the tray. Use several small dollops of silicon gel to secure the wire to the tray and to seal any small gaps around the male banana plug fitting. Fill each tray with 100 ml of 0.13 M Tris-citrate buffer (pH 7.0) and connect them to a power supply (we use ISCO Model 493 Electrophoresis Power Supply units) by electrical leads fitted with female banana plugs.

Place a gel, loaded with samples, on a block of "blue ice" between two trays. Allow one end of a cellulose sponge (moistened with electrode buffer) to overlap the gel 0.5 cm from the line of wicks and place the other end of the sponge in the electrode buffer reservoir. Repeat on the opposite end of the gel, allowing one end of the sponge to overlap the gel by 2 cm. This completes the circuit across and through the gel.

Dip a pair of forceps into a 1% methylene blue solution and insert the tip of the forceps into the gel 1 cm from the edge of the mold and just below the cellulose sponge which is opposite the wicks. Use the mobility of the dye relative to the mobility of the enzyme-activity bands to calculate R_f values.

Place a piece of plastic wrap over the gel and sponges. Then place a piece of PVC board on top of the gel to ensure good contact between the

sponges and the gels. Set a second, smaller block of reusable "blue ice" on top of the PVC board to maintain a constant temperature of 4 C throughout electrophoresis. Replace the ice as necessary. Electrophorese the gels at constant voltage.

Enzyme-activity Staining: Following electrophoresis, slice the gel horizontally into several slices (depending on the gel thickness). The slicing surface we use consists of a piece of PVC board with two raised edges (3 mm). Our gel slicer is a modified coping saw with a 0.009 mm guitar string stretched tightly across one end. For staining ACO, LAP, MPI, and IDH, we remove and discard both the surface and the bottom layers. We then stain the remaining center slice for enzyme activity. For other enzymes, we discard only the surface layer. Place the gel slice into a Pyrex baking dish containing the stain solution for a specific enzyme or protein. The staining recipes we use have been adapted from a number of published sources, including Shaw and Prasad (1969), Brewbaker et al. (1968), O'Malley et al. (1980), Vallejos (1983), Cardy et al. (1980) and others.

The sections that follow outline buffer formulations, gel preparation methods, and enzyme-activity staining protocols.

I. Gel and Electrode Buffer Formulations

A. Gel Buffer: 0.005 M L-Histidine (pH 7.0)

L-Histidine (HCl)	1.048 gm
4 N NaOH	1.1 ml
Distilled water	1.0 liter (final volume)

Dissolve chemicals in distilled water and adjust pH with 4N NaOH.

B. Electrode Buffer: 0.13 M Tris-Citrate (pH 7.0)

Tris	15.73 gm
Citric acid	7.68 gm
Distilled water	1.0 liter (final volume)

Dissolve chemicals in distilled water and adjust pH with conc. HCl.

II. Gel Preparation

The following summarizes the methods for single layer gel preparations. Two- and-three layer gels are made by modifying the amounts of chemicals and buffers as outlined in Gel types. NOTE: Many of the chemicals used to make gels are toxic. Exercise caution, wear protective clothing and work in a well-ventilated area.

a) 12.5% (w/v) starch

0.005 M Histidine (HCl)	240 ml
Hydrolyzed potato starch (Sigma Electrophoresis Grade)	30 gm

Combine the starch and buffer in a 1000 ml Erlenmeyer side-arm flask containing a large magnetic stirring bar. Stopper the flask and place it in a hot water bath on a magnetic stirrer/hot plate. Heat the starch solution with constant stirring to 80 C. Then add any cofactors (e.g., NADP, NAD, etc.) and allow them to mix into the heated starch solution. Degas the flask via vacuum aspiration for 40 seconds and pour immediately into a 2-layer gel mold. (Note: Because starch gels often are brittle, we do not make these gels in single-layer gel molds). Slowly lower a piece of plate glass (20 x 18 x 0.6 cm) onto the gel to produce an even surface.

b) 7% (w/v) acrylamide

0.005 M L-Histidine (HCl)	150 ml
Acrylamide	9.975 gm
N,N'-methylene-bis-acrylamide	0.525 gm
APS	0.15 gm
TEMED	0.30 ml

Add all the chemicals except TEMED to the gel buffer in a 1000-ml glass beaker, mix well and heat to 30 C with constant stirring. Then add TEMED to the mixture and quickly pour the solution into a single-layer gel mold.

c) 9% (w/v) acrylamide

0.005 M L-Histidine (HCl)	150 ml
Acrylamide	12.82 gm
N,N'-bis-acrylamide	0.675 gm
APS	0.15 gm
TEMED	0.30 ml

The method for preparing this gel is the same as for (b).

d) 7% (w/v) acrylamide + 2% (w/v) starch

0.005 M L-Histidine (HCl)	150 ml
Acrylamide	9.975 gm
N,N'-bis-acrylamide	0.525 gm
APS	0.15 gm
TEMED	0.30 ml
Hydrolyzed potato starch	3.0 gm

Add acrylamide chemicals, APS, and any cofactors to one half of the total volume of gel buffer and mix well with constant stirring at room temperature. Combine the starch and remaining volume of gel buffer in an Erlenmeyer side-arm flask, mix well, stopper the flask and heat (with constant stirring) to 80 C. Then degas the solution for 40 second. Add the hot starch solution to the acrylamide solution and mix thoroughly. Then add TEMED and quickly pour the solution into a single-layer gel mold.

e) 6% (w/v) acrylamide + 4% (w/v) starch

0.005 M L-Histidine (HCl)	150 ml
Acrylamide	8.55 gm
N,N'-methylene-bis-acrylamide	0.45 gm
APS	0.15 gm
TEMED	0.30 ml
Hydrolyzed potato starch	6.0 gm

The preparation of this gel is the same as described for (d).

Allow gels to cool to room temperature. For gels b-e, pour off any unpolymerized solution on the surface. Wrap the gels in plastic wrap (except a) and refrigerate at least eight hours before use.

III. Stain Buffer Formulations

The volume of distilled water in the following buffer formulations represent the final volume of the solution.

A. 0.2 M Tris-HCl (pH 8.5)

Tris	24.22 g.
Distilled water	1000 ml

Dissolve Tris in distilled water and adjust pH with concentrated HCl.

B. 0.1 M Acetate Buffer (pH 5.0)

- A. 0.2 M Acetic acid (11.55 ml in 1000 ml dHOH)
- B. 0.2 M Sodium acetate (16.4 gm $C_2H_3O_2Na$ in 1000 ml dHOH)

Add 148 ml A + 352 ml B and bring up to 1000 ml with dHOH.

C. 0.1 M Phosphate Buffer (pH 6.4)

Sodium monophosphate	13.9 gm
Sodium diphosphate	5.3 gm
Distilled water	1000 ml

Dissolve chemicals in distilled water and adjust pH with 1 M HCl.

D. 0.1 M Tris-Maleate Buffer (pH 5.2)

Tris	12.1 gm
Maleic anhydride	11.6 gm
Sodium hydroxide	1.6 gm
Distilled water	1000 ml

Dissolve chemicals in distilled water and adjust pH with 0.2 M HCl.

E. 0.2 M Tris-Maleate Buffer (pH 3.7)

0.2 M Tris	24.2 gm
0.2 M Maleic acid	23.2 gm
Distilled water	1000 ml

Dissolve chemicals in distilled water and adjust pH. To use as a stain buffer, dilute the stock as follows: 5 (stock) : 3 (dHOH) : 2 (0.2 M NaOH). Final pH = 5.55.

F. 0.01 M Malate Buffer (pH 7.0)

L-Malic acid	1.341 gm
Trizma base	3.025 gm
Distilled water	1000 ml

Dissolve the chemicals in distilled water and adjust pH with 4 M NaOH.

G. 0.05 M Sodium Phosphate Buffer (pH 7.0)

- A. 0.05 M Monobasic sodium phosphate
(6.9 gm NaH_2PO_4 in 1 Liter dHOH)
- B. 0.05 M Dibasic sodium phosphate
(7.1 gm $\text{Na}_2\text{H}_2\text{PO}_4$ in liter dHOH)

39 ml A + 61 ml B. Adjust pH with 1 M HCl.

H. 0.05% Aniline Blue Solution

Aniline blue	0.05 gm
7% acetic acid	100 ml

Dissolve the aniline blue in the 7% acetic acid.

I. Potassium Iodide Solution

Iodine	0.10 gm
Potassium iodide	0.50 gm
Distilled water	100 ml

Dissolve the chemicals in distilled water in a covered beaker. It may take several hours for the iodine to dissolve. Store in an amber bottle.

IV. Enzyme-activity stain protocols

Gel electrophoresis times depend on gel thickness and laboratory conditions. Many of the chemicals used in the staining process are toxic and must be handled and disposed of with extreme caution.

A. OXIOREDUCTASES

1. Alcohol Dehydrogenase (ADH) EC 1.1.1.1

Gel: D + 15 mg NAD

Time: 8 hours at 200 volts

0.05 Sodium phosphate buffer	50 ml
MTT	15 mg
NAD	15 mg
95% Ethanol	5 ml
PMS	2 mg

Place the gel in the dark at room temperature for 1 hour.

2. Diaphorase (DIA) EC 1.6.2.2

Gel: D

Time 12 hours at 200 volts

0.2 ml Tris-HCl	50 ml
MTT	10 mg
NADH	10 mg
2,6 Dichlorophenol indophenol	2 mg

Incubate the gel at 37 C in the dark for 2 hours.

3. Glucose 6-phosphate dehydrogenase (GPD) EC 1.1.1.49

Gel: E + 15 mg NADP

Time: 24 hours at 150 volts

0.2 N Tris-HCl	50 ml
MTT	10 mg
NADP	15 MG
Na ₂ Glucose 6-phosphate	100 mg
PMS	1 mg

Incubate the gel at 37 C in the dark for 3 hours.

4. Isocitrate dehydrogenase (IDH) EC 1.1.1.42
(NADP-active)

Gel: D

Time: 12 hours at 200 volts

0.2 M Tris-HCL	50 ml
MTT	10 mg
NADP	10 mg
MgCl ₂	120 mg
DL-Isocitric acid	200 mg
PMS	1 mg

Incubate the gel at 37 C in the dark for 3 hours.

5. Malate dehydrogenase (MDH) EC 1.1.1.37

Gel: D + 15 mg NAD

Time: 8 hours at 200 volts

0.01 M Malate buffer	50 ml
MTT	15 mg
NAD	30 mg
PMS	1 mg

Incubate the gel at 37 C in the dark for 1 hour.

6. Peroxidase (PER) EC 1.11.1.7

Gel D

Time: 8 hours at 200 volts

3-amino-9-ethylcarbazole	
(dissolved in 1.25 ml dimethyl formamide)	25 mg
CaCl ₂	1 ml
H ₂ O ₂ (3% solution)	1 ml
Sodium acetate	3 ml
Distilled water (chilled)	46 ml

Add chemicals slowly to prevent reprecipitation of 3-amino-9-ethylcarbazole. Incubate the gel in the dark at room temperature for 1 hour.

7. 6-Phosphogluconate dehydrogenase (PGD) EC 1.1.1.43

Gel: E

Time: 16 hours at 150 volts

0.2 M Tris-HCl	50 ml
MTT	10 mg
NADP	10 mg
MgCl ₂	20 mg
6-phosphogluconic acid	12 mg
PMS	1 mg

Incubate the gel at 37 C in the dark for 1 hour.

8. Shikimate dehydrogenase (SKD) EC 1.1.1.25

Gel: E + 15 mg NADP

Time: 24 hours at 150 volts

0.2 M Tris-HCl 50 ml

MTT 15 mg

NADP 15 mg

Shikimic acid 15 mg

PMS 1 mg

Incubate the gel at 37 C in the dark for 1 hour.

B. TRANSFERASES

9. Glutamate oxaloacetic transaminase (GOT) EC 2.6.1.1

Gel: D

Time: 13 hours at 200 volts

0.2 M Tris-HCl 50 ml

Pyridoxal 5'-phosphate 25 mg

L-aspartic acid 272 mg

Ketoglutaric acid 36 mg

Fast Blue BB salt 112 mg

Incubate the gel at 37 C in the dark for 1 hour.

10. Phosphoglucomutase (PGM) EC 2.7.5.3

Gel: D

Time: 8 hours at 200 volts

0.02 M Tris-HCl 50 ml

MTT 10 mg

NAD 10 mg

MgCl₂ 20 mgNa₂ D-glucose 1-phosphate 125 mgGlucose 6-phosphate dehydrogenase
(NAD active) 40 units

PMS 1 mg

Incubate the gel at 37 C in the dark for 2 hours.

C. HYDROLASES

11. Acid phosphatase (ACP) EC 3.1.3.2

Gel: D

Time: 12 hours at 200 volts

0.2 M Acetate buffer 50 ml

Black K salt 40 mg

Alpha-naphthyl acid phosphate 40 mg

Place the gel at room temperature in the dark.

12. Beta-Amylase (AM) EC 3.2.1.2

Gel: B

Time: 4 hours at 200 volts

A. 0.2 M Acetate buffer	100 ml
Soluble potato starch	1 gm

Heat the potato starch in buffer A until dissolved. Cool the solution to 30 C and pour over the gel. Incubate the gel at 37 C in the dark for 15 to 30 minutes. Pour off A and rinse the gel with distilled water.

B. 0.1% iodine in 0.5% potassium iodine.

Pour 20 ml of B over the gel. Score bands immediately.

13. Endopeptidase (ENP) EC 3.4.???

Gel: D

Time: 12 hours at 200 volts

0.2 M Tris-Maleate pH 5.5	50 ml
Black K salt	20 mg
MgCl ₂	10 mg
N-alpha-Benzoyl DL-arginine	
beta-naphthylamide (HCl)	20 mg
(BANA)	

Stir stain solution vigorously for 10 minutes in the dark and then pour over the gel. Place the gel at room temperature in the dark for 2 hours.

14. Esterase (EST) EC 3.1.1.1

Gel: E

Time: 6 hours at 200 volts

0.1 M Phosphate buffer	50 ml
Fast Blue RR salt	100 mg
Alpha-naphthyl butyrate	1 mg
100% Acetone	3 drops
1% Alpha-naphthyl acetate	1 ml

Add alpha-naphthyl butyrate and three drops of acetone to the Fast Blue RR salt. Then add 50 ml of phosphate buffer and stir the solution vigorously over low heat. Add 1 ml of 1% alpha-naphthyl acetate to the warm solution. Pour the solution immediately over the gel through a single layer of cheesecloth. Incubate the gel at 37 C in the dark for 2 hours.

15. Fluorescent esterase (FLE) EC 3.1.1.2

Gel: D

Time: 12 hours at 200 volts

0.2 M Acetate buffer	50 ml
100% Acetone	10 mg
4-methylumbelliferyl acetate	15 mg

Dissolve 15 mg of 4-methylumbelliferyl acetate in 100% acetone. To this solution add 30 ml of acetate buffer. Saturate three Kim-wipes with the stain solution and place in direct contact with the sliced gel surface. Examine the gel with a UV light (366nm) source soon after staining as the bands fade quickly.

16. Leucine aminopeptidase (LAP) EC 3.4.11.1

Gel: D

Time: 8 hours at 200 volts

A. 0.1 M Tris-Maleate buffer 100 ml
(25 ml stock + 75 ml dHOH)
L-leucine-beta-naphthylamide
(dissolved in 3 drops 100% acetone) 20 mg

Incubate the gel in A at 37 C for one hour. Then pour A into a beaker for use in next step.

B. Black K salt 500 mg

Add B to A and pour back over gel. Place the gel at room temperature for 1 hour.

17. Urease (EU) EC 3.5.1.5

Gel: D

Time: 8 hours at 200 bolts

A. 0.2 M Acetate buffer 100 ml
0.1% (w/v) Cresol red 0.1 gm

Pour A over the gel and place gel at room temperature in the dark for 10-15 minutes. Pour off A.

B. 333 mM Urea 2 gm
0.1% (w/v) Cresol red 0.1 gm
0.1% (w/v) Na₂EDTA 0.1 gm
Distilled water 100 ml

Pour B over the gel. Place the gel at room temperature in the dark. Bands appear within 15 minutes. Record results immediately as the bands blur within a few hours.

D. LYASES

18. Aconitase (ACO) EC 4.2.1.3

Gel: A + 15 mg NADP

Time: 6 hours at 160 volts

0.2 M Tris-HCl 50 ml
MTT 10 mg
NADP 10 mg
MgCl₂ 10 mg
cis-Aconitic acid
(1% solution, pH 7.5) 8 ml
Isocitrate dehydrogenase 40 units
PMS 1 mg

Incubate the gel at 37 C in the dark for 3 hours.

19. Mannose 6-phosphate isomerase (MPI) EP 5.3.1.8

Gel: D

Time: 8 hours at 200 volts

0.2 M Tris-HCl	50 ml
MTT	10 mg
NAD	15 mg
Mannose 6-phosphate	20 mg
Glucose 6-phosphate dehydro- genase (NAD active)	40 units
Phosphoglucose isomerase	40 units
PMS	1 mg

Incubate the gel at 37 C in the dark for 1 hour.

20. Phosphoglucose isomerase (PGI) EC 5.3.1.9

Gel: D

Time: 8 hours at 200 volts

0.2 M Tris-HCl	50 ml
MTT	10 mg
NAD	10 mg
MgCl ₂	20 mg
Fructose 6-phosphate	30 mg
Glucose 6-phosphate de- hydrogenase	40 units
PMS	1 mg

Incubate the gel at 37 C in the dark for 1 hour.

F. PROTEIN

21. Kunitz Trypsin inhibitor (Ti)

Gel: C

Time: 4 hours at 200 volts

A. 0.05% Aniline blue solution

Pour aniline blue solution over gel and let stand undisturbed for five minutes. Pour off B (can be reused several times).

B. 7% Acetic acid

Pour acetic acid over the gel and replace as it acquires a blue tint. Allow the gel to destain overnight.

Abbreviations:

NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (reduced)
NADP	Nicotinamide adenine dinucleotide phosphate
MTT	4,5-dimethylthiozoyl-2)-2,5 diphenyltetrazolium bromide
PMS	Phenazine methosulfate
TRIS	Tris(hydroxymethyl)aminomethane
Na EDTA	Ethylenediaminetetraacetate (disodium salt)

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1) Soil manganese stress in soybean.

Introduction: Growth-limiting factors associated with soil acidity include toxicities of manganese and other metal ions, low pH (H^+ toxicity), and deficiencies or unavailabilities of essential elements such as Ca, Mg, Mo, and P (Foy, 1983). Acid soil factors may act independently, or together, to affect the growth of plants. Manganese toxicity is one of the major growth-limiting factors in many acid soils. Manganese toxicity generally occurs in soils having pH values of 5.5 or below, if the soil parent materials contain sufficient total Mn (Foy, 1973, 1981.) Plant species and cultivars differ widely in their tolerances to excess soluble or exchangeable Mn (Foy, 1983). Corn and rice are more tolerant than soybean or barley. Excess Mn affects plant tops more directly than roots (Foy, 1973; 1982a). For a given plant, Mn accumulates somewhat in proportion to plant injury (Osawa and Ikeda, 1980). The objective of this study was to determine the sensitivity or tolerance of various vegetable soybean genotypes to high level of soil manganese and low soil pH.

Materials and methods: A Hayesville sandy loam soil was used for the study in a greenhouse. The soil was characterized with respect to texture, pH, cation exchange capacity (CEC), organic matter, and extractable Mn. The soil was divided into two batches, and one batch was adjusted to pH 6.3 with $CaCO_3$. The soil was allowed to equilibrate for a few weeks to stabilize the pH level and determined. The soil pH levels used for the experiment were pH 5.2 (original soil pH) and 6.3. Then, soil was placed in 15-cm diameter plastic pots and 1 g per pot of 5-10-10 N-P-K fertilizer applied to provide the major nutrients. A split plot design with three replications of each treatment was utilized. pH levels of the soil were the whole plot treatments and soybean genotypes were subplot treatments. Thirty seven vegetable soybean genotypes belonging to different maturity groups (III to VIII) were tested in the study. The plants were allowed to grow to the dry pod stage. At initiation of flowering, fully developed leaves were collected for Mn analysis. At dry pod stage, plants were harvested at ground level and pods were collected for determination of seed yield. The data obtained were subjected to statistical analysis by using analysis of variance and Duncan's multiple range test.

Results and discussion: Genotypes PI 417128, PI 417213, PI 417440, PI 423758, 'Hahto', 'Kingston', 'Peking', 'Rokusun' and some others were very tolerant to high level of soil Mn and low soil pH, and their seed yield was unaffected under the conditions (Table 1). Genotypes PI 417288, 'Funk Delicious', 'Kahala', 'Mokapu Summer', 'Sango', 'Verde', and 'Wilson 5' were sensitive to high level of soil Mn and low soil pH and their seed

Table 1. Vegetable soybean genotypes sensitive or tolerant to high soil manganese and low soil pH, reflected in yield difference.

Genotype	Soil pH 5.2 (High soil Mn)	Soil pH 6.3 (Low soil Mn)	TI* index
PI 423758	7.39	5.14	144
Kingston	3.03	2.18	139
PI 417440	5.82	4.32	135
Aoda	6.60	4.99	132
Rokusun	5.26	4.26	123
Hahto	7.26	6.47	112
PI 417213	5.64	5.25	107
PI 417128	6.37	6.29	101
Peking	2.92	2.92	100
Hahto/Michigan	6.75	6.78	100
Kanrich	4.98	5.20	96
PI 222397	5.21	5.49	95
PI 416982	6.30	6.72	94
KaiKoo	5.24	5.79	90
Willomi	5.78	6.43	90
Oakland	5.25	5.85	90
Sato	5.94	6.62	90
Wolverine	5.82	6.51	89
Pella	6.03	6.83	88
PI 417359	6.44	7.43	87
Williams 79	5.58	6.56	85
Kura	6.63	7.86	85
Kailua	5.77	6.99	82
PI 417159	5.63	7.07	80
PI 416771	3.79	5.02	75
PI 416893	4.51	6.02	75
Kim	5.70	7.64	75
Shiro	6.64	8.93	74
Emperor	4.27	5.80	74
Wave	4.30	6.02	71
Mokapu Summer	5.26	7.55	69
Kahala	4.37	6.29	69
Funk Delicious	4.73	6.86	69
Sango	5.03	7.40	68
Wilson 5	2.77	4.23	65
Verde	3.52	5.77	61
PI 417288	3.16	5.93	53

*Tolerance Index >90 very tolerant; 70-89 tolerant; 50-69 sensitive.

yield decreased at pH 5.2. According to analysis of variance, there was a significant different ($P < 0.01$) in seed yield between the genotypes. Overall, the soil pH effect was significant ($P < 0.01$). There was no significant interaction between genotypes and pH levels, as for the seed yield. In general, Mn concentration was higher in the soybean leaf tissue at pH 5.2, compared with pH 6.3. The genotypes that were sensitive to Mn did not tolerate the high level of Mn in the plant tissue and resulted in reduced seed yield at pH 5.2. The genotypes with high concentration of Mn in leaf tissue and unaffected seed yields at pH 5.2 appear to tolerate excess Mn. In conclusion, the genotypes found to be tolerant to Mn toxicity could be utilized in breeding programs for development of better vegetable soybean cultivars for problem soils.

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1) Aluminum tolerance of soybean callus cultures: Comparison with greenhouse and solution culture screening methods ✓✓

Soybean is generally not tolerant of high aluminum (Al) saturation. In North Carolina, soybean yield on highly Al saturated, unlimed soils averaged 70% of that obtained on limed soil (Dunphy and Schmitt, 1981). Although the existence of genetic variability for Al tolerance has been documented in soybean (Foy, 1984), selection of Al-tolerant cultivars has been hindered by lack of efficient, reliable screening methods.

A number of researchers have used cell culture methods to identify and select for Al tolerance in plant species including soybean (Conner and Meredith, 1984; Smith et al., 1983; Meredith, 1978; Ojima and Ohira, 1983; Zakri, 1986). If soybean tolerance to Al is primarily a cellular, rather than a morphological phenomenon, then Al-tolerant genotypes may be identifiable using a cell culture approach. The advantages of a cell culture approach are (1) genotypes may be subjected to uniform Al stress in the culture media, and, (2) cell culture can induce sources of tolerance through somatic mutation. In some species, these mutants can be regenerated; however, a relationship between Al tolerance at the cellular level and tolerance of whole plants has not been reported. Sain and Johnson (1986) have noted that two soybean genotypes with contrasting Fe efficiency were similarly efficient or inefficient in cell culture.

The objectives of this research were (1) to develop and test a method for ranking soybean genotypes for Al tolerance in callus culture and (2) to relate genotypic rankings for Al tolerance at the cellular level with greenhouse and solution-culture methods of evaluating whole plants.

Materials and methods: Twelve genotypes were selected to represent a range of differential tolerance to Al. These included 'Essex', N77-114, PI 381674, and PI 416937 from maturity group IV; 'Jeff', 'Sable', N80-2177-2, PI 319529, and PI 424391 from maturity group VI; and 'Gasoy 17', WH3-27, and FC 31732 from maturity group VII. These genotypes were compared in the following screening methods:

Callus culture screen: The basal culture medium (D-5R) was modified from MS (Murashige and Skoog, 1962) as follows: N reduced to 30 mM comprised of 20.9 mM NO_3 and 8.7 mM NH_4 , K reduced to 13.5 mM, and Zn increased to 85 μM . The screening medium, NONH, was developed from D-5R. Modifications were as follows: NO_3 decreased and NH_4 increased to 15 mM each so that upward drift of pH over the culture period was reduced, Ca decreased to 1.5 mM to minimize possible ameliorative effects of Ca on Al toxicity, and EDTA removed to inhibit formation of Al-EDTA chelates. The initial pH after autoclaving was 4.6. A portion of NONH medium also contained 2.5 mM Al as $\text{Al}_2(\text{SO}_4)_3$. Four callus pieces, each 130-160 mg in fresh weight, were placed on each petri plate, one genotype per plate. The experiment

was arranged as a randomized complete block with six replications in a factorial combination of the Al vs. the control treatment and 12 genotypes. The plates were incubated at 25 C under a 14-h photoperiod for 10 days beginning on 4 Nov. 1987. After the incubation period, fresh weights were determined for the four individual calli per plate and averaged prior to statistical analyses.

Greenhouse screen: The control treatment consisted of a Goldsboro sandy loam with an initial Al saturation of 19% and pH of 4.7. A portion of this soil was amended with $\text{Al}_2(\text{SO}_4)_3$ to achieve an Al saturation of 55% and pH of 4.0 for the Al treatment. A split-plot experimental design was employed with the control and Al treatments assigned to whole plots and the 12 genotypes to subplots. Seven days after planting (13 July, 1987), the 12-L pots were thinned to two plants of the test genotype. Plants were grown under extended photoperiod (14-h) to prevent flowering, watered as needed, and fertilized with 250 mg K, 130 mg P, and 600 mg N/pot (Nutrileaf soluble fertilizer, Miller Chem. Co., Hanover, PA) in split applications. Forty days after planting, tops were harvested and subsequently dried at 70 C. Dry weights were determined on a per plant basis.

Solution culture screen: This screen was similar to that of Hanson and Kamprath (1979) except the control treatment consisted of a 200 μM CaSO_4 solution and the Al treatment was a solution of 200 μM CaSO_4 + 7-15 μM $\text{Al}_2(\text{SO}_4)_3$. The experiment was arranged as a split plot with six replications over time, beginning on 13 Aug., 1987. Whole plots were assigned to control and Al solutions, subplots were the genotypes. Each genotype was represented by 16 seedlings per treatment solution per replication. Radical growth, calculated as the Relative Extension Rate (Sartan and Kamprath, 1978), was averaged over the 16 seedlings per vat.

Statistical analyses: For the purpose of evaluating Al tolerance, growth in the Al treatment was expressed and statistically analyzed as a percentage of the control. Genotypic relationships among the screening methods were determined by considering each method as a separate testing environment and performing analysis of variance over multiple environments. Estimates of genotypic and genotype x method mean squares thus obtained were used to calculate genotypic correlations after Eisen and Saxton (1983).

Results and discussion: Al tolerance was estimated both as growth in highly Al-saturated soil per se and as percent-of-control because these expressions are most often chosen by breeders for selection purposes in artificial screening environments (Table 1). When growth was expressed as percent-of-control, the genotypic correlation between the greenhouse and solution culture methods was high. In contrast, the association between callus culture and the other screening methods was disappointingly low (Table 2). Although similar rankings were indicated for some genotypes as callus and as whole plants, other genotypes were strikingly divergent.

In soybean, 100-seed-weight has been positively associated with both seedling root elongation and seed yield at harvest (Devine et al., 1979;

Tekrony et al., 1987). In our study, 100-seed-weight for the genotypes ranged from 12.4 to 23.9 g, and was positively associated with Al tolerance for the greenhouse and solution-culture screens. Genotypes with larger seeds tended to be more tolerant (Table 1). In contrast, no association was found between seed weight and genotypic means in the callus culture screen. This result may be explained because increased seed reserves would not be expected to influence growth of established callus.

In the greenhouse screen, early plant vigor associated with seed reserves (weight of germinating seed) probably increased seedling tolerance to stress. Adjusting the greenhouse results for genetic differences in initial seed weight removed some of the morphological influences on Al tolerance and thus drastically improved the correlation between the greenhouse whole plant and cell culture screening methods (from $r = 0.19$ to $r = 0.64$). This provides a strong indication that the callus-culture screen could be used to identify biochemical mechanisms of Al tolerance. When easily repeatable techniques for soybean regeneration are developed, selection of novel forms of biochemical resistance to Al may be possible in callus culture. Currently, the callus culture screen may be best suited to the study of those existing genotypes that possess Al tolerance mechanisms operating at the cellular level. Further studies are required to establish the relationship between the three screening methods tested here and the field environment.

Table 1. Mean seed weight and Al tolerance of 12 soybean genotypes as measured in three Al screening methods. Al tolerance is expressed as growth in Al per se and as percent-of-control (PC)

Genotype	100-seed weight g	Greenhouse		Solution		Callus	
		Top dry wt.		RER §		Fresh wt.	
		Al	PC	Al	PC	Al	PC
		g	%		%	mg	%
PI 416937	23.0 [†]	4.12 [‡]	95	0.235	74	387	63
WH3-27	19.1	5.19	87	0.237	70	374	53
PI 319529	14.4	4.55	84	0.233	70	325	75
FC 31732	23.9	4.50	83	0.234	71	360	41
Gasoy 17	13.8	3.61	73	0.197	68	362	58
N77-114	17.1	3.63	69	0.192	61	353	48
Jeff	15.7	3.09	67	0.219	70	375	55
Sable	16.2	2.76	67	0.242	68	425	53
N80-2177-2	17.4	3.49	63	0.195	63	443	62
PI 424391	12.4	3.60	59	0.166	63	363	64
Essex	14.5	3.27	54	0.181	61	356	43
PI 381674	17.2	2.95	53	0.200	69	309	67
S.E. [¶]	1.4	0.90	38.3	0.015	5.7	43.4	8.2

§ RER = Relative extension rate calculated as $[\ln(\text{final root length}) - \ln(\text{initial root length})]/\text{days in solution}$.

[†] Based on 200 seed.

[‡] Means were based on six observations.

[¶] S.E. = standard error from analyses of variance.

Table 2. Genotypic and phenotypic correlations between Al tolerance rankings for 12 soybean genotypes as measured in three screening methods. Tolerance is expressed as growth in Al per se and as percent-of-control (PC).

Method 1	vs.	Method 2	r_G^s	
			Al	PC
Greenhouse [†]		Solution [‡]	0.49	0.94
Greenhouse		Callus [¶]	-0.19	0.24
Solution		Callus	0.19	0.33
S.E.			0.11	0.12

§ Genotypic correlations calculated from analyses of variance where each method was considered a separate environment.

[†] The trait measured in the greenhouse screen was top dry weight.

[‡] RER = Relative extension rate calculated as $[\ln(\text{final root length}) - \ln(\text{initial root length})]/\text{days in solution}$.

[¶] The trait measured in the callus culture screen was fresh weight.

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 2) Effect of temperature on expression of the impermeable seed coat trait. 3

The presence of an impermeable seed coat inhibits or delays the imbibition of water by the seed. Selection within soybean populations that are segregating for the impermeable trait is complicated by variable expression of permeability levels in different field environments (Tinius and Hinson, 1987; Tinius et al., 1988). To facilitate the isolation of genotypes that produce discrete, repeatable levels of seed coat permeability, it would be helpful to identify those environmental factors that contribute most to variable expression of the trait. Baciú-Miclaus (1970) found that low relative humidity and high temperatures from anthesis to beginning maturity increased the percentage of impermeable seeds in field-grown soybean. Evidence of a differential effect of temperature on permeability was demonstrated by Potts et al. (1978). They showed that the percentage of impermeable seeds from an impermeable soybean breeding line increased as drying temperature increased, while the percentage of impermeable seeds for the permeable cultivar 'Dare' was unchanged. In a large sample of soybean genotypes with various levels of seed coat permeability, mean percentage of impermeable seeds was closely associated with both temperature and rainfall during the months of seed formation and maturation (Tinius, unpublished). The objective of this study was to evaluate the influence of temperature on expression of the impermeable trait in controlled environments, using closely related genotypes with different levels of seed coat permeability.

The study was conducted in the Southeastern Plant Environment Laboratories (Phytotron) at North Carolina State University. The soybean genotypes used originated as selections from the cross ('Kirby' x ('Forrest' (3) x D77-12480)) x F81-5590. The impermeable seed coat trait was donated by F81-5590, which was selected from the cross D65-8232 x D77-12480. The trait was carried in D65-8232 and traces to PI 163453 (Glycine soja Sieb. & Zucc.). D77-12480 is a selection from 'Tracy' x ('Hill' x PI 159925), and is the source of the 'long juvenile' trait donated by PI 159925, which delays flowering under short-day conditions (Cregan and Hartwig, 1984). All plants used in the study exhibited the long juvenile response. Two pairs of near-isolines that represented a wide range of seed coat permeability were used for the experiment. Each pair was selected from an F7 hill plot grown at Hartsville, SC, in 1987, and both pairs were selected from the same F6 hill in 1986 at Clayton, NC. Each of the four genotypes was seeded into 6-liter plastic pots that contained the standard Phytotron gravel and peat-like mix. At 10 days after planting, each pot was thinned to one plant/pot.

Plants were grown in controlled environment "A" chambers set at 26 C day/22 C night with a 9 h photoperiod and were watered twice daily with the Phytotron nutrient solution. Two "A" chambers were used to establish

two temperature regimes: 18 C day/14 C night, and 30 C day/26 C night. These temperature regimes approximate field conditions at Raleigh, NC (cool) and Gainesville, FL (warm) when adapted soybeans normally mature. At growth stage R5 (Fehr and Caviness, 1977) plants were moved into the different temperature regimes. Four plants of each genotype were allocated to each temperature regime in a completely random design, and the experiment was conducted twice.

In both experiments, all plants were harvested singly at growth stage R8 and maintained at temperatures approximating their respective temperature treatments in the Phytotron. Seeds were threshed manually to minimize scarification. Samples of 25 to 100 seeds from each plant were tested for permeability by soaking in petri dishes that contained germination paper and deionized water. Only seeds that had no obvious defects were tested, and the number still impermeable at 1 and 27 h in water was recorded. Any plant that failed to produce at least 25 seeds that could be tested was excluded from the analyses. All data were arcsine square root transformed before the analyses were performed. Least squares means and standard errors were computed for each temperature genotype combination. Single degree of freedom contrasts were conducted to test differences between genotypes in each pair of near isolines.

None of the genotypes produced seeds that were impermeable at 27 h. Furthermore, many of the seeds from the warm temperature regime were not tested, due to damaged seed coats. The combined analyses showed that there was a significant ($P < 0.05$) increase in impermeability when genotypes matured under warm temperatures (Table 1). Overall, differences due to genotypes were non-significant, as was the temperature x genotype interactions. However, when data for each pair of near-isolines were analyzed separately, there was a significant ($P < 0.01$) temperature x genotype interaction for the lines 47-161 and 47-162 (Table 2).

The results of this study agree with observations from field experiments that temperature during seed formation and maturation affects the expression of the impermeable seed coat trait. Additionally, there was further evidence of a differential response of genotypes to different temperature regimes, which suggests that a given level of permeability may be variably expressed in different environments. Thus, selection of discrete levels of seed coat permeability would be dependent on a particular environment, and such levels may not be widely adapted. Hill et al. (1986) have shown that high soil moisture availability during seedfill will reduce the expression of impermeability by disrupting seed coat integrity. Plants in the Phytotron had high soil moisture throughout the experiment, and many seed coats were badly ruptured. Further studies are needed to characterize the dual effects of temperature and soil moisture and to correlate results from field experiments in order to develop a predictive model for impermeable seed coat expression over a range of environments.

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Table 1. Means and standard errors of percentage of impermeable seeds at 1 h soak for two temperature regimes.

Temperature regime	Mean %
18 C day/14 C night	5.8 \pm 1.8
30 C day/26 C night	12.0 \pm 2.1

Table 2. Means and standard errors of percentage of impermeable seeds at 1 h soak for four genotypes.

Near-isoline pair	Genotype	Temperature regime	
		18 C day/14 C night	30 C day/26 C night
		-----%	
1	47-161	5.0 \pm 3.6	4.7 \pm 4.6
	47-162	1.2 \pm 3.9	16.2 \pm 3.9
2	48-216	5.0 \pm 3.6	8.8 \pm 4.2
	48-217	11.8 \pm 3.6	18.3 \pm 4.2

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1) Analysis of the Yl8 gene of soybean and its associated alleles,

An unstable allele has been described at the Yl8 locus of soybean (Peterson and Weber, 1969). This nuclear-encoded gene contributes toward proper development of the chloroplasts. Three alleles have been identified: Yl8 - the dominant allele conditions normal green chloroplasts; yl8 - the recessive allele conditions defective yellow chloroplasts; and Yl8-m - the mutable allele conditions green/yellow variegated leaves. Genetic analysis has revealed that in addition to the observed somatic mutability produced by the mutable allele, germinal mutations also occur such that either pure breeding green (rare) or pure breeding yellow (common) plants can be recovered from the mutable stock. The yellow plants are generally lethal; however, they can be grown to maturity under reduced light conditions (i.e., in a greenhouse or growth chamber). An ultrastructural analysis of the defective chloroplasts from yellow tissue has been performed (Palmer et al., 1979). Isolated grana stacks, separation of the lamellae and an increased number of osmiophilic bodies per chloroplast were observed when compared to normal chloroplasts. Also, the chlorophyll content was reduced by 50%, but the ratio of chlorophyll a and b remained the same.

The instability of the Yl8-m allele is affected by temperature (Sheridan and Palmer, 1977). This observation coupled with the genetic data (Peterson and Weber, 1969) strongly suggests the interaction of a transposable element system with the Yl8 locus to produce the instability of expression of the Yl8-m allele. When compared with transposable element systems from other plants (Vodkin, 1988), however, Yl8-m exhibits what has been termed "reverse variegation". In other words, the unstable allele reverts from a dominant to a recessive form and not vice versa, as is typical in most other systems. Therefore, an understanding of this mutation would be quite informative in regards to the nature and action of transposable elements.

With this in mind, attempts are being made to better understand the mutation from both a biochemical and molecular perspective. The following is a summary of observations thus far:

1. Three different shadings of green can be observed on either the upper or lower leaf surfaces of mottled leaves (green, light green, very light green). These shadings reflect mutations to yl8 in individual cell layers in the leaf (Palmer et al., 1979). More detailed observations indicate the mutations to yl8 can be restricted to the upper palisades layer, to both palisades layers, or to the spongy

mesophyll. Mutations restricted to the lower palisades layer have not been observed. In addition, all cell layers can be involved in the mutation event to produce yellow leaves. This mutant, therefore, may prove useful in studies of leaf development through clonal analysis as has been demonstrated in corn.

2. All previous evidence suggests that the direction of mutation for the unstable allele occurs in one direction only: namely, from green to yellow and not in reverse (Peterson and Weber, 1969; Sheridan and Palmer, 1977). However, recent observations suggest that the mutation from yellow to green can occur. This is seen somatically in rare instances where green leaf sectors are surrounded by yellow tissue. Also, two greenhouse-grown plants have been identified that suggest germinal mutation events in the direction of yellow to green. Both plants developed at least to the stage of the first trifoliolate as fully yellow plants before producing green or mottled leaf axillary branches. Seed derived from these branches produced progeny that segregated for leaf color phenotype indicating a mutation event in the germinal cell layer.
3. Initial biochemical and molecular characterization of the defect conditioned by the y18 allele at the Y18 gene has begun. Polyacrylamide gel electrophoresis of thylakoid fractions from chloroplasts isolated from green and yellow tissue revealed the absence of six polypeptides in the yellow chloroplast extracts. Missing are polypeptides of the following estimated sizes: 51 kD, 35.5 kD, 30.5 kD, 23.5 kD, 20.5 kD and 17.5 kD. In addition, total RNA has been extracted from green and yellow leaf tissue. Visualization on agarose/formaldehyde gels reveals a quantitative reduction in the rRNAs of the chloroplasts. Analysis of in vitro translation products produced from mRNA isolated from the total RNA is in progress to identify polypeptides affected by Y18.
4. Since Tgml exhibits the typical structural features of a transposable element (Vodkin et al., 1983), attempts were made to associate instability of Y18-m with mobility of Tgml or Tgml-related elements. DNA was extracted from green and yellow leaf tissue and analyzed on Southern blots, using a Tgml internal 1.3 kb Hind III fragment as a probe. Several different restriction enzymes were used and no banding pattern differences were observed between the two DNA preparations. This suggests that rearrangements of Tgml are not associated with the Y18-m instability.

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1) Genetic linkage analysis for 34 pairs of loci./

Genetic linkage tests were performed both in the greenhouse and in the field for a total of 34 pairs of genes. The genetic traits indicated by the gene symbols in Table 1 are described by Palmer and Kilen (1987). Recombination was calculated from F2 data using the product method as described by Immer and Henderson (1943). No evidence of linkage was found except in the combinations e and t, f and rj1, and t and yl2. Evidence of independent assortment of loci is needed for distinguishing linkage groups associated with distinct chromosomes. Weiss (1970) previously reported that e1 and t were linked (linkage group 1) with a recombination value of 3.9 ± 0.4 centimorgans and that yl2 and t were linked (linkage group 1) with a recombination value of 21.6 ± 0.7 centimorgans. Devine et al. (1983) reported the linkage of f and rj1 (linkage group 11) with a recombination value of 40.0 ± 2.2 centimorgans.

The linkage values calculated from the current data for f and rj1 and t and yl2 are in good agreement with previous reports. The difference in the recombination value of 3.9 ± 0.4 centimorgans for e1 and t reported by Weiss (1970) and the value of 7 ± 1.3 centimorgans found in our test may be due to environmental effects or to genetic differences among parental genotypes in factors affecting recombination frequencies. It is also possible that an additional locus controlling maturity, distinct from e1, is located in close proximity to the e1 locus.

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Table 1. Soybean linkage test.

Cross	Genes	A	B	C	D	Sum	%R	S.E.	Phase
1) T162 x BARC-1	<u>e</u> , <u>f</u>	253	94	84	18	449	42	4	R
2) T162 x BARC-1	<u>e</u> , <u>rj1</u>	249	88	91	22	450	45	4	R
3) T162 x BARC-1	<u>e</u> , <u>t</u>	325	14	15	96	450	7	1.3	C
4) T162 x BARC-1	<u>e</u> , <u>y17</u>	265	98	67	20	450	53	4	C
5) T162 X BARC-1	<u>f</u> , <u>rj1</u>	369	101	97	47	614	42	2.8	C
6) T233 x BARC-1	<u>f</u> , <u>rj1</u>	237	85	59	44	425	39	3	C
<u>Combined data</u>	<u>f</u> , <u>rj1</u>	606	186	156	91	1039	41	2.1	C
7) T162 x BARC-1	<u>f</u> , <u>t</u>	359	111	109	35	614	51	3	C
8) T233 x BARC-1	<u>f</u> , <u>t</u>	252	70	86	17	425	45	4	R
<u>Combined data</u>	<u>f</u> , <u>t</u>	611	181	195	52	1039	49	2.3	R
9) T162 x BARC-1	<u>f</u> , <u>y17</u>	372	98	116	28	614	49	3.1	R
10) T233 x BARC-1	<u>f</u> , <u>y12</u>	244	78	85	18	425	44	4	R
11) Minsoy x BARC-2 (Rj4)	<u>fr1</u> , <u>rj4</u>	156	54	41	14	265	50	4.6	C
12) T145 x PI 290136	<u>fr2</u> , <u>p1</u>	764	237	267	81	1349	50	2	C
13) PI 290136 x BARC-4 (Rj2)	<u>fr2</u> , <u>rj2</u>	132	55	43	14	244	53	5.0	C
14) PI 290136 x BARC-2 (Rj4)	<u>fr2</u> , <u>Rj4</u>	167	61	56	19	303	51	4.4	C
15) PI 290136 x BARC-2 (Rj4)	<u>fr2</u> , <u>w1</u>	84	29	26	10	149	48	6.0	C
16) BARC-4 (Rj2) x BV-1	<u>p1</u> , <u>rj2</u>	224	59	62	23	368	55	4	R
17) T162 x T145	<u>p1</u> , <u>w1</u>	275	83	137	53	548	53	3.1	R
18) BARC-4 (Rj2) x BV-1	<u>p1</u> , <u>y10</u>	213	68	73	14	368	43	4	R
19) T162 X T145	<u>p1</u> , <u>y17</u>	500	152	164	38	854	54	2.7	C
20) T31 x Clark <u>rj1</u>	<u>p2</u> , <u>rj1</u>	157	36	24	30	247	I+	4.5	R
21) T162x T31	<u>p2</u> , <u>y17</u>	602	218	203	47	1070	44	2.4	R
22) T141 x T135	<u>pc</u> , <u>y9</u>	127	58	48	20	253	49	5	R
23) T141 x T230	<u>pc</u> , <u>y13</u>	127	58	48	20	253	49	5	R
24) T162 x BARC-1	<u>rj1</u> , <u>t</u>	353	115	113	33	614	48	3.1	R
25) T233 x BARC-1	<u>rj1</u> , <u>t</u>	239	57	99	30	425	53	4	R
<u>Combined data</u>	<u>rj1</u> , <u>t</u>	592	172	212	63	1029	50	2.3	R
26) T162 x BARC-1	<u>rj1</u> , <u>y17</u>	369	120	94	31	614	50	3	R
27) T233 x BARC-2	<u>rj1</u> , <u>y12</u>	234	62	95	34	425	54	3	R
28) BARC-4 (Rj2) x BV-1	<u>rj2</u> , <u>y10</u>	218	65	63	22	368	48	4	C

1. Soybean linkage test. (cont'd)

Cross	Genes	A	B	C	D	Sum	%R	S.E.	Phase
29) T230 x BARC-4 (<u>Rj2</u>)	<u>rj2</u> , <u>y13</u>	270	91	58	31	450	44	3.3	C
30) T162 x BARC-4 (<u>Rj2</u>)	<u>rj2</u> , <u>y17</u>	132	35	28	11	206	44	4.9	C
31) PI 290136 x BARC-2 (<u>Rj4</u>)	<u>rj4</u> , <u>w1</u>	73	28	40	8	149	I+	6.5	C
32) Combined with 1984 data	<u>rj4</u> , <u>w1</u>	305	107	119	25	556	I+	3.4	C
33) T233 x BARC-2 (<u>Rj4</u>)	<u>rj4</u> , <u>y12</u>	218	55	99	15	393	I+	4	C
34) T162 x BARC-2 (<u>Rj4</u>)	<u>rj4</u> , <u>y17</u>	659	206	168	74	1107	45	2	C
35) T162 x BARC-1	<u>t</u> , <u>y17</u>	369	119	99	27	614	52	3.1	C
36) T233 x BARC-1	<u>t</u> , <u>y12</u>	448	49	45	83	625	18	1.7	C
37) T162 x T145	<u>w1</u> , <u>y17</u>	273	85	148	42	548	49	3.2	R

+I = Independent, %R>55

Lines prefixed by T are from the soybean genetic type collection (Palmer and Kilen, 1987) maintained by R. L. Bernard, ARS, USDA, Agron. Dept., Univ. of Illinois. PI 290136 is from the plant introduction collection obtained from the same source. BV-1 is a line constructed at Beltsville from T161 and T145 carrying P1 and y10. BARC-1 carrying f and rj1, BARC-2 (Rj4) carrying Rj4 and BARC-4 (Rj2) carrying Rj2 were constructed at Beltsville and described in Crop Science 26:1091, Crop Science 26:1263-1264 and Crop Science 27:1322-1323, respectively.

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1) Genetic gain by stages in regional soybean tests.

Introduction: Development of improved cultivars requires testing in multiple stages. Typically, a large number of genotypes are entered in the first stage. Selection results in progressively smaller numbers of entries as stages progress, culminating in the release of a small number of genotypes as cultivars. Testing is usually the most expensive item in the breeder's budget, and the breeder must decide how to allocate limited resources to each stage of the process. The objective of this research was to examine results derived from the final stages of a soybean breeding program, i.e., cooperative regional tests, to obtain estimates of selection differentials, genetic gain, and realized quasi-heritabilities for quantitative traits.

Materials and methods: Results of the Uniform Regional Soybean Tests, Northern States, from 1960 to 1987 provided the data for this study. These were tests of experimental soybean lines conducted cooperatively by researchers in the USA and Canada. The stages selected for study were (1) the preliminary tests, which were the first regional tests for lines developed in individual breeder's programs and were usually conducted at 7 to 12 locations, and (2) the first year of the uniform tests, which were usually conducted at 7 to 30 locations. Test data were distributed to participating breeders, and selection of lines was based on participants' votes. Selected lines from the preliminary tests were evaluated the following year in uniform tests; selections made among lines in their first year in uniform tests were evaluated a second year in the uniform test. There were tests in 6 maturity groups (00 to IV), but preliminary tests in groups 00 and 0 were discontinued in the 1970's.

Check cultivars common to the test in which selections were made and the following year's uniform test, where selections were re-evaluated, were used to adjust data for year effects and obtain estimates of genetic gain. The characters studied were yield (as a percentage of the common checks), days to mature, lodging, plant height, seed size, seed protein concentration, and seed oil concentration. Yield expressed as kg/ha was also studied, but the results were very similar to those obtained for yield as a percentage of the checks and are therefore not presented. The selection differential (I) was estimated by $I = X_{si} - X_i$, where X_{si} and X_i are the means of selected and all experimental entries, respectively, in the test at stage i . Genetic gain (ΔG) was estimated by $\Delta G = (X_{su} - X_{cu}) - (X_i - X_{ci})$, where X_{ci} is the mean of the common checks in the test at stage i , and X_{su} and X_{cu} are means of the selected entries and common checks, respectively, in the following year's uniform test. Realized quasi-heritability (h^2) was estimated by regressing ΔG on I with the constraint that the regression line must pass through the origin ($\Delta G = Ih^2$). (The term quasi-heritability is used here because heritability

Table 1. Estimates of mean selection differential (upper figure), genetic gain (middle figure), and realized quasi-heritability (lower figure) for selection from regional trials, 1960-86.

Maturity Gp.	00	I					II					III					IV					pooled	
		0		I		UT	II		UT	III		UT	IV		UT	V		UT	VI		UT	pooled	
		UPT	UT	UPT	UT		UPT	UT		UPT	UT		UPT	UT		UPT	UT		UPT	UT		UPT	UT
No. tests	11	21	24	27	24	27	29	23	31	24	28	25	141	141									
Yield	4.3*	2.1*	2.4*	3.9*	3.7*	2.4*	4.1*	2.0*	6.7*	3.0*	7.0*	2.6*	5.2	2.7									
(% check)	-0.2	-0.4	2.1	3.3*	2.2*	2.2*	2.2	0.3*	3.4*	2.1*	4.7*	2.6*	3.0	1.5									
	0.19	-0.24	0.15	0.98	0.58	0.58	0.58	0.62	0.55	0.62	0.68	1.11	0.61	0.66									
Maturity	-0.8	0.1	-0.4	-0.8*	0.3	-0.7*	-0.7*	0.4	-0.4	0.5	0.2	0.1	-0.5	0.2									
(days)	-0.8	0.7	-0.3	-0.6	0.5	-0.7*	-0.7*	0.3	-0.6*	0.4	0.3	0.2	-0.4	0.4									
	0.52	0.41	0.92	0.82	0.94	0.94	0.99	1.10	0.86	1.06	0.90	0.85	0.84	0.86									
Lodging	-0.03	-0.03	-0.09	-0.03	0.00	-0.03	-0.03	-0.04	-0.13*	-0.04	-0.11*	0.05	-0.08	-0.02									
(score) ²	0.09	0.01	-0.01	-0.05	0.02	0.02	0.00	-0.06	-0.11*	-0.04	-0.06	0.10	-0.04	0.01									
	0.18	0.81	0.77	0.81	0.79	0.79	0.79	1.00	0.76	0.92	1.02	1.28	0.80	0.95									
Height	-0.5	0.7	-0.2	-0.9	1.0	1.0	0.2	0.4	-2.0	1.4	1.5	2.0	-0.3	0.9									
(cm)	-0.2	-0.3	-0.6	-0.5	0.8	-0.7	-0.1	-0.2	-0.8	1.5	0.4	2.5	-0.3	0.6									
	0.90	0.43	1.23	0.75	1.12	1.12	1.03	0.92	0.92	1.05	0.91	0.94	0.93	0.95									
Seed	-0.15	0.02	0.06	0.17	0.05	0.05	0.24	0.00	0.19	-0.01	0.24*	0.16	0.17	0.05									
size	0.10	0.45*	-0.06	0.11	-0.02	-0.02	0.03	-0.19	0.15	-0.07	-0.01	0.13	0.06	0.04									
(cg/seed)	0.50	1.12	1.14	0.92	0.84	0.84	0.89	0.99	0.96	0.95	0.93	0.86	0.91	0.98									
Protein	-0.10	-0.08	-0.13	-0.03	0.02	0.02	-0.15	-0.11	-0.30*	-0.04	-0.06	-0.06	-0.14	-0.06									
(%)	-0.25	-0.08	-0.25	0.07	-0.03	-0.03	-0.13	-0.04	-0.20	0.10	0.00	-0.07	-0.10	-0.04									
	1.08	0.73	0.66	1.11	0.96	0.96	0.82	0.51	0.62	0.43	1.18	0.86	0.87	0.79									
Oil	-0.10	0.12	0.12	0.18*	0.24*	0.24*	0.19*	0.07	0.22*	-0.02	0.12	-0.02	0.15	0.06									
(%)	0.02	-0.01	0.05	0.18*	0.26	0.26	0.16	-0.02	-0.20*	0.02*	0.18	-0.02	0.15	0.04									
	0.03	0.62	0.37	0.93	1.11	1.11	1.31	0.92	0.81	0.88	0.88	0.86	0.91	0.93									

* Significantly different ($P=0.05$) from zero by t-test.

¹ UPT=selections made in Preliminary Test, gain determined from performance of selections in following year's Uniform Test; UT=selections made among lines in first year of Uniform Test; gain determined from performance in following year's Uniform Test.

² scored from 1 (erect) to 5 (prostrate).

strictly applies to a parameter estimated using a random sample from a well defined reference population.)

Results and discussion: Selection differentials indicated that yield was the primary selection criterion, with early maturity and lodging resistance important in preliminary tests in some maturity groups (Table 1). Selection differentials and genetic responses for higher oil and lower protein occurred in some maturity groups, perhaps reflecting correlations with yield. In absolute value, selection differentials and genetic gain were smaller for the first-year uniform tests than for preliminary tests, except for maturity and height.

Quasi-heritabilities were generally high, except for yield. They were similar between the preliminary tests and the first-year uniform test.

Despite selection differentials similar to those of other maturity groups, the tests in maturity group 00 produced relatively little gain for yield. Low quasi-heritabilities were evident for yield in preliminary test 0 and for several traits in both group 00 tests. Schutz and Bernard (1967) reported low among-strain variances for yield in regional tests of early lines. Breeders working in the early maturity groups may be poorly served by selection based on regional means.

Mean yield gains were 3.0% and 1.5% for preliminary tests and first-year uniform tests, respectively. Gains made at any stage of a multi-stage testing program are influenced by gains in previous stages (Finney, 1966; Young, 1976). This makes it difficult to reach conclusions about the effectiveness of programs in the absence of data on each stage. Analysis of all stages of a breeding program by methods similar to those used here may suggest ways to improve efficiency.

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1) Green pod yield and architectural traits of selected vegetable soybean genotypes.

During the 1988 growing season, an experiment was conducted at Randolph Research Farm, Virginia State University, Petersburg, Virginia, to determine the associations between the vegetable soybean architectural traits and green pod yield. Seventeen vegetable soybean genotypes ranging in maturity from IV to VI were planted in a randomized complete block design, with three replications. The architectural traits and green pod yield and its components were determined by harvesting the soybean genotypes at different reproductive (R5, R6, and R7) stages (Fehr et al., 1971).

The initial study indicated that significant differences for all traits studied were observed among genotypes. Significant genotype x reproductive stages interactions were also observed. This indicates that genetic variability exists among the genotypes tested for further selection and hybridization. The green pod yield of the genotypes ranged from 895 to 2056 g/m² (Table 1). The cultivar 'Kingston' produced the lowest and PI 423.759 produced the highest green pod yield. The high green pod yield observed in PI 423.759 is probably attributed to the high number of pods/plant observed.

The phenotypic correlations observed between traits arising from the combined effects of genotype and environment is presented in Table 2. Significant and positive phenotypic correlations were observed between green pod yield and the two yield components: 1) number of pods/plant and 2) 100-pod weight. In contrast, the correlation between 100-pod weight and number of pods/plant was negative. Therefore, selection for increased pods/plant should result in correlated response for increased green pod yield, but 100-pod weight would be reduced. All architectural traits studied, except branches/plant had significant positive phenotypic correlation with green pod yield. The phenotypic correlations indicated that architectural traits are not independent, and that selection for increased levels of expression of one architectural trait will likely result in concurrent changes in the level of expression of others.

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Table 1. Mean of architectural traits, green pod yield, and yield components of 17 vegetable soybean genotypes.

	Architectural traits					Yield components		
	Bran- ches/ plant	Nodes/ branch	Nodes on main stem	Main stem height	Main stem inter- node length	Green pod yield	Pods/ plant	100-pod weight
	-----	No.-----		----cm.-----		--g/m ² --	-No.-	--g---
Ware	5.69	1.82	8.89	27.16	2.98	1057.41	50.66	79.40
Emperor	6.59	2.50	10.06	39.53	3.92	1350.68	56.74	110.31
Sango	2.58	3.64	12.84	55.96	4.38	1582.34	40.74	112.80
Kingston	7.54	1.41	8.89	24.91	2.77	895.16	71.57	38.41
Sooty	2.42	5.91	17.51	73.80	4.22	1478.42	79.70	59.00
Wilson-5	4.91	3.41	13.81	67.79	4.88	1170.38	75.74	55.35
PI 416.982	7.63	2.12	12.54	42.56	3.35	1601.30	40.41	133.41
PI 417.288	4.83	1.84	12.06	38.96	3.22	1818.60	51.33	163.40
PI 417.322	7.68	2.18	11.36	38.08	3.35	1306.44	45.30	110.84
PI 416.771	8.58	1.59	9.57	24.77	2.52	1236.96	79.10	65.30
PI 417.052	8.21	2.24	13.78	58.81	4.43	1333.83	78.28	49.25
PI 423.759	7.98	2.21	13.92	48.82	3.65	2055.55	149.78	42.45
PI 417.213	9.26	1.74	12.49	46.73	3.74	1375.11	57.17	116.13
PI 417.310	10.83	2.86	13.09	42.03	3.20	1711.54	80.321	95.96
PI 171.437	7.24	3.59	14.71	46.22	3.12	1349.37	123.90	29.80
PI 423.852	9.37	2.13	14.04	40.83	2.91	1987.34	115.26	67.82
PI 222.397	5.48	4.92	21.63	71.46	3.68	1736.79	61.64	67.82
LSD (0.05)	1.80	1.42	2.20	14.47	0.61	638.56	30.06	21.89

Table 2. Simple linear correlation coefficients between different architectural traits, green pod yield, and yield components of 17 vegetable soybean genotypes.

Architect- ural traits/ green pod yield components	Traits							
	Number of branches/plant	Number of pods/branch	Nodes/main stem	Number of pods/plant	Main stem height	Main stem internode length	100-pod weight	Green pod yield
					-cm-	-cm-	-g-	g/m ²
Number of branches/ plant	--	0.523**	-0.127	0.441**	-0.289**	-0.382**	-0.031	0.157
Number of nodes/ branch		--	0.446**	0.526**	0.251**	-0.078	-0.129	0.267**
Number of nodes/main stem			--	0.246**	0.759**	0.223**	-0.134	0.285**
Number of pods plant-				--	0.094	-0.136	-0.414**	0.257*
Main stem height (cm)					--	0.715**	-0.017	0.311*
Main stem internode length (cm)						--	0.121	0.243**
100-pod weight (g)							--	0.409*

*,** Correlation coefficient significantly different from zero at 0.50 and 0.01 level, respectively.

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1) Cold tolerance during reproductive growth of soybean✓

Soybean is a quite warm-requiring plant. To ensure normal growth and development of the plants, depending upon variety, a sum of temperatures above 15 C 1600-3200 C is needed (Enken, 1959). Early ripening varieties developed over the last years are able to mature and provide high yields when the sum of active temperatures is 1600-2000 C. Soybean is, in particular, sensitive to temperature in the period of seed germination, flowering, and bean formation. Enken (1959) considers temperatures of 12-14 C to be enough for seed germination. The most favorable conditions in the period of flowering and bean formation are 20-25 C. Lowering of temperature in this period often results in yield reduction. Therefore, studies of different aspects of cold resistance at different stages of ontogenesis are being intensively carried out both in our country and abroad. As a result of such work, the genotypes that can develop normally at low plus temperatures in the period of seed germination and flowering stage have been revealed (Sichkar, 1981, 1984; Sherepitko and Balashov, 1985; Sichkar and Beversdorf, 1982; Barashkov et al., 1982; Malysh and Riasantseva, 1982; Malysh and Bobrikov, 1984; Lawn and Hume, 1985; Littlejohns and Tanner, 1976; Saito et al., 1970; and Szirmer and Yanicka, 1985).

Soybean varieties developed in the north forest steppe zone of the Ukraine developed normally and formed beans at 18-20 C. But even short-term temperature lowering, which does not occur often, resulted in considerable disturbance of fertilization; hence it leads to reduction in yield of many varieties and prolongation of the vegetative period.

At the same time, there are varieties of soybean that can produce stable high yield even under such conditions. The available genetic diversity for reaction to low temperatures makes it possible for breeders to work at creation of the varieties that are cold resistant during all ontogenetic stages.

The main purpose of this work was to study the reaction to low temperatures at flowering period in quite contrasting years of some soybean varieties, which were used in further experiments as parental components. Mean temperatures of the air during the flowering period of the studied varieties in June and July, 1983, were 17.8 and 18.7 C, respectively; in 1984 they were 15.2 and 17.2 C. In 1983, at flowering, during only one day, temperature lowered to 11 C, and during three days up

to 12 - 13 C. So we consider air temperature in 1983 to be, on the whole, normal for flowering and bean formation. In 1984, the number of days when mean temperature at flowering did not exceed 13 C averaged 20; on some days it lowered to 9.5 C, and at definite period of a day--up to 3-4 C. All those factors had a negative effect on fertilization in many varieties; therefore, their productivity was greatly reduced. The described conditions made it possible to assess exactly soybean varieties in their reaction to lowered temperatures during the flowering period. Data from Table 1 show that, in 1984, with low temperatures at flowering, the number of seeds per pod, and 1000-seeds weight appeared to be the more stable characters. The greatest reduction in comparison with 1983 was observed in the number of pods and seeds per plant, by 36-75% and 55-80% lower, respectively. This resulted in considerable decrease of total seed productivity. Therefore, those characters can be considered criteria of cold hardiness of soybean genotypes at flowering. Taking into consideration that the number of pods per plant is a subcomponent of more complicated characters, such as "number of seeds per plant" and "seed weight per plant", this rather common character was taken as a criterion of soybean cold hardiness at flowering.

Nevertheless, absolute values of the character cannot be used directly for cold resistance analysis of soybean genotypes, because initial genotypes significantly differ in development of the character under optimum conditions as well. That is why the degree of cold resistance was estimated as a percentage of the number of beans per plant yielded in a cold 1984, to the number of beans per plant of the corresponding soybean variety in 1983. Table 1 shows that "Iskra" variety demonstrated worse cold resistance at flowering period than other varieties, in particular Canadian line "419211-0225" and "Nordic" variety.

In this connection, variety Iskra was used for studying cold resistance inheritance as the maternal variety in hybridization, and pollen was taken for pollination from other varieties.

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Table 1. Index of main components of productivity of soybean plants in 1984 in comparison with 1983.

Cultivar	Index				
	Number of pods/plant	Number of seeds/plant	Weight of seeds/plant	Number of seeds/pod	Wt of 1000 seeds
Iskra	24.9 \pm 1.5	19.1 \pm 1.3	17.0 \pm 1.1	81.4 \pm 2.2*	92.8 \pm 1.7
419211-0225	64.2 \pm 6.7	60.7 \pm 0.1*	60.2 \pm 6.1	97.5 \pm 2.7*	95.9 \pm 1.4
VNIIMK 9186	38.2 \pm 3.1*	44.3 \pm 3.1*	33.3 \pm 2.4*	121.6 \pm 4.0*	76.2 \pm 1.9*
Nordic	52.5 \pm 4.5*	52.9 \pm 4.7*	54.7 \pm 4.7*	100.9 \pm 3.8*	104.6 \pm 2.4*

* significant at the P=0.05 level.

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2) The inheritance of cold resistance in soybean//

Revealing the genetic dependence of resistance to low temperatures in different periods of plant growth and development will enable successful breeding of cold-resistant varieties. Moldavian scientists demonstrated that inheritance of cold resistance at the first stages of ontogenesis was controlled by one main dominant gene (Sherepitko and Balashov, 1985). The genetics of soybean resistance to low temperatures during flowering period has not been studied enough.

In this connection, cold-sensitive Iskra variety was crossed with cold-resistance varieties 419211-0225, 'VNIIMK 9186' and 'Nordic'. The F₁ was grown in 1983. Evaluation of the F₂ plants was done in the field in 1984, when strong negative influence of low temperatures in the period of flowering and bean formation was observed.

Distribution of the plants with different levels of cold resistance is shown in Table 1, and the average of cold resistance and variations of the character in studied populations are shown in Table 2. These data demonstrated that all paternal varieties essentially exceed the maternal variety in resistance to cold. High phenotypic variation of this trait caused the overlap of some part of the variation series and for VNIIMK 9186--all classes of variation series, made for the plants of parental varieties and the F₂. Under such conditions, the analysis of F₂ segregation is quite difficult, identification of plant genotypes according to phenotype without special marker genes is impossible. Therefore the attempt was made to analyze by biometrical methods the available experimental material at the phenotypic level, not trying to distinguish in the F₂ homozygotes and heterozygotes according to "cold resistance at flowering period". For this purpose we shall calculate theoretically supposed values of plant frequencies with pronounced cold resistance level for maternal variety Iskra, and cold resistance demonstrated by 419211-0225, VNIIMK 9186, and Nordic.

First of all, it should be noted that the classes of variation series with "cold resistance" from 0 to 60 include practically all plants of maternal variety. This is demonstrated not only by the picture of distribution of the frequencies upon the classes of variation series, but also by average value and mean square deviation (S), from what it follows that plants with cold resistance within the limits from 0 to 60% make up 99.5% of the population of Iskra plants (Table 2). From now we shall call the plants with such expression of the studied characters "maternal phenotype" and others with cold resistance >60% as "paternal phenotype". It should be taken into consideration that different parts of paternal plants have "maternal phenotype" (Table 3).

The absence of analogous information, concerning the studied character in plants --heterozygotes (i.e., essentially the F₁ plants), which are included in the F₂ populations together with parental homozygotes,

complicates further analysis. Thus, to calculate theoretically supposed segregation of the F2 into plants with "maternal" and "paternal" characters based on the suggestion that differences in the character are conditioned by one gene, it is necessary to determine the parameters of the F1 population for each cross.

It is known (Mather and Jinks, 1985) that the F2 differs from average parental value (m) by a value which is equal to half of F1 deviation from average parental value:

$$\bar{F}_2 = m + \frac{1}{2} / \bar{F}_1 - m/, \text{ from which it follows that } F_1 = 2F_2 - m.$$

Found from this formula an average number of beans per plant for the F1 populations, and already known from a field experiment values of the character for the F1 plants (1983), made it possible to determine the degree of cold resistance for groups of heterozygous plants (i.e., F1) for each cross. For the F1 of Iskra x 419211-0225 it was 78.7%, Iskra x VNIIMK 9186 - 58.9%, and Iskra x Nordic - 65.7%. Now we can determine what part of the F1 plants, i.e., heterozygotes in 1984, will be presented by plants with "maternal" expression of the character. So we can estimate on the basis of the known characteristics of initial parental varieties and F2 the regression coefficient (b) of "the percentage of plants with maternal type of cold resistance" (y_i) in relation to "cold resistance degree of plants" (x_i)

$$b = \frac{\sum /x_i - \bar{x}/ \quad /y_i - \bar{y}/}{\sum /x_i - \bar{x}/^2} = -1.53$$

Thus, we found that the increase of average value of cold resistance in population by 1%, resulted in 1.5% decrease in number of nonresistant plants (maternal phenotype). Then we substitute in the regression equation $y_i = \bar{y} + b/x_i - \bar{x}/$ for \bar{y} value (arithmetic average of plant percentage with maternal expression of cold resistance in all parents and F2), b value (regression coefficient), \bar{x} (arithmetic average of plant cold resistance in all parents) and $F2/$, x_i (arithmetic average of plant cold resistance in the studied population)¹. Hence, we can find y_i (percentage of plants with maternal expression of the character in the corresponding population). For the F1 of Iskra x 419211-0225, the given index was 27.3%, for Iskra x VNIIMK 9186 - 58.1%, and for Iskra x Nordic - 47.7%.

Now we have all the information to calculate theoretically segregation of the F2 of each cross according to "cold resistance of soybean plants at flowering period", coming from the hypothesis of monohybrid segregation. Let us consider the parental plants to be presented by the next genotypes: Iskra - aa, 419211-0225 - A1A1, VNIIMK 9186 - A2A2, Nordic - A3A3.

Then segregation in the F2 will be expressed in the ratios of 7.5 aa : 15 Aa : 7.5 AA, while analyzing 30 plants of each cross. To move on from genotypes ratio to the supposed ratio of phenotypes with maternal and

paternal expression of the character, it is necessary to take into account unequal degrees of expression of the A1, A2, and A3 genes both in homozygous and heterozygous states. It is necessary to take into account previously calculated percentage of "maternal phenotype" plants, which appears among the plants of different parents. For instance, in the F2 of cross Iskra x 419211-0225, the supposed ratio of progenies with maternal expression of cold resistance to the paternal one will be 100% from 7.5 aa + 27.3% from 15 Ala + 45.4% from 7.5 AlAl, whereas the number of plants with paternal expression of the character will make up 72.7% from 15 Ala + 54.6% from 7.5 AlAl. After calculations, we shall receive the ratio of 15 plants with maternal expression of the character : 15 plants with paternal expression.

In the same way, the theoretically supposed ratio of phenotypes in the F2 for two other crosses was established. Correspondence between the theoretical segregation and the actual one was tested by Chi-square value (Table 4). It is shown that the possibility of correspondence between the actual segregation and monohybrid segregation is high in all three crosses. That is why there are no reasons to reject the hypothesis of monohybrid segregation of cold resistance in the F2.

Thus, the differences in cold resistance at flowering between Iskra variety and the paternal varieties 419211-0225, VNIIMK 9186 and Nordic are conditioned by one gene. Heterosis by cold resistance was observed in the F1 of all tested crosses.

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Table 1. Level of resistance to low temperature in soybean crosses and parents.

Parent, cross	Variation series of cold resistance to low temperature							
	N	0-20	21-40	41-60	61-80	81-100	101-120	121-140
Iskra	63	28	29	6				
419211-0225	22	1	6	3	5	4	2	1
F2 Iskra x 419211-0225	30	1	6	7	11	2	2	1
VNIIMK 9186	25	1	14	10				
F2 Iskra x VNIIMK 9186	30	4	8	12	4	1	1	
Nordic	27	2	6	11	5	2	1	
F2 Iskra x Nordic	30	1	7	14	7	1		

Table 2. Cold resistance of the parents and the F2

Parent, cross	N	$\bar{Y} \pm \frac{S_y}{\sqrt{N}}$	S	CV
Iskra	63	24.9 ± 1.5	11.9	47.8
419211-0225	22	$64.2 \pm 6.7^*$	31.3	48.7
F2 Iskra x 419211-0225	30	$61.8 \pm 5.0^*$	27.6	44.7
VNIIMK 9186	25	$38.2 \pm 3.1^*$	15.3	40.0
F2 Iskra x VNIIMK 9186	30	$45.0 \pm 3.9^*$	21.2	47.1
Nordic	27	$52.5 \pm 4.5^*$	24.0	45.7
F2 Iskra x Nordic	30	$52.3 \pm 3.5^*$	19.4	51.5

* - significant at the $P = 0.05$ level.

Table 3. Number of plants with "maternal phenotype" in the parents and the F2. 1984.

Cross, parent	Number of studied plants	Number of plants with "maternal phenotype"	Percentage of plants with "maternal phenotype"
419211-0225	22	10	45.4
F2 Iskra x 419211-0225	30	14	46.7
VNIIMK 9186	25	25	100
F2 Iskra x VNIIMK 9186	30	24	80
Nordic	27	19	70.4
F1 Iskra x Nordic	30	22	73.3

Table 4. Analysis of distribution of cold resistance in the F2

	F		O-E	O-E	$\frac{O-E}{E}$	Proba- bility
Phenotype	Actual Observed	Theoret. Expected				
Iskra x 419211-0225						
Susceptible	14	15	-1	1	0.066	
Resistant	16	15	+1	1	0.066	
	30	30			X = 0.132	0.75-0.50
Iskra x VNIIMK 9186						
Susceptible	24	23.7	0.3	0.09	0.004	
Resistant	5	6.3	-0.3	0.09	0.015	
	30	30			X = 0.019	0.90
Iskra x Nordic						
Susceptible	22	19.45	2.55	0.50	0.33	
Resistant	8	10.55	-2.55	0.50	0.62	
	30	30			X = 0.95	0.50-0.25

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